The Ecology and Biology of Stingrays (Dasyatidae) at Ningaloo Reef, Western Australia

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Submitted by

Owen R. O'Shea BSc (Hons I)

School of Biological Sciences and Biotechnology
Murdoch University, Western Australia

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Declaration

I declare that this thesis is my own account of my research and contains as its main content, work that has not previously been submitted for a degree at any tertiary education institution.

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Owen R. O'Shea **Date**

Publications Arising from this Research

- **O'Shea, O.R.** (2010) New locality record for the parasitic leech *Pterobdella amara*, and two new host stingrays at Ningaloo Reef, Western Australia. *Marine Biodiversity Records* 3 e113
- **O'Shea, O.R.,** Thums, M., van Keulen, M. and Meekan, M. (2012) Bioturbation by stingray at Ningaloo Reef, Western Australia. *Marine and Freshwater Research* 63:(3), 189-197
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- **O'Shea, O.R.,** Braccini, M., McAuley, R., Speed, C. and Meekan, M. (In review). Optimising the estimation of growth parameters of difficult-to-sample species: an example for tropical stingrays. *PLoS One*
- Speed, C.W., **O'Shea, O.R.** and Meekan, M.G. (2013). Transmitter attachment and release methods for short-term shark and stingray tracking on coral reefs. *Marine Biology* DOI 10.1007/s00227-012-2151-y
- Cerutti-Pereyra F, Meekan MG, Wei NWV, **O'Shea O.R.**, Bradshaw CJA, Austin CM (2012) Identification of Rays through DNA Barcoding: An Application for Ecologists. *PLoS one* 7:e36479
- Kempster, R.M., Garza, E., Egeberg, C.A., Hart, N.S., **O'Shea, O.R**. and Collin, S.P. (In review) Sexual dimorphism in the electrosensory system: a quantitative analysis of nerve axons in the dorsal anterior lateral line nerve of the fantail stingray *Taeniura lymma* (Forsskål 1775). *Frontiers in Neuroanatomy*

Conference Presentations Arising from this Research

- **Australian Marine Science Association (AMSA), July 2011, Fremantle, Western Australia**
  Bioturbation by stingrays at Ningaloo Reef, Western Australia

- **International Coral Reef Symposium (ICRS), July 2012, Cairns, Queensland, Australia**
  Physical and biological effects associated with stingray foraging behaviour at Ningaloo Reef, Western Australia

- **Australia and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) July 2012, Perth, Western Australia**
  Lethal sampling of stingrays for scientific research

- **American Elasmobranch Society (AES) August 2012, Vancouver, Canada**
  Physical and biological effects associated with stingray foraging behaviour at Ningaloo Reef, Western Australia

Abstract

Batoids make up a significant portion of the biomass in coastal and nearshore ecosystems, yet few data are available on the functional role and life history characteristics of rays in these environments. Given their conservative life history traits and vulnerability to extrinsic pressures, urgent information is required to further understand this little known group of fishes. The objectives of this research were to assess the biological and ecological characteristics of tropical stingrays at Ningaloo Reef, Western Australia. More specifically, I wanted to quantify the physical and biological impacts associated with predation by stingrays, prey specificity and trophic resource partitioning and age and growth of five sympatric species (*Himantura uarnak, Neotrygon kuhlii*, *Pastinachus atrus*, *Taeniura lymma* and *Urogymnus asperrimus*). A technical assessment for safe and ethical lethal sampling protocols for large dasyatid rays is discussed as a foundation to the research that was conducted for this degree. Strict codes of practice for the welfare of animals in scientific research demand up to date methodologies for ethical consideration, especially where death is an endpoint. Safe and humane techniques were developed as part of this study in order to sample the rays required using lethal methods. These techniques proved successful with both considerations met and it is hoped, will provide a framework for safe practices for any future work where lethal sampling of large, potentially hazardous demersal elasmobranchs is required. Age and growth parameter estimates were evaluated for these five species by sectioning and counting calcium band-pair deposition in vertebral samples. Due to less than ideal sample sizes on account of logistical constraints, a multi-analytical approach was adopted to optimise parameter estimates and generate

realistic results. This included using a Bayesian framework to approximate the posterior distribution of the growth parameters. Growth rates of smaller-bodied species were faster than for larger-bodied species, but longevity was shorter. The oldest recorded age from these samples was 27 years and although validation was not possible, annual deposition is assumed based on previous accounts of similar species. This is the first time that growth parameter estimates in dasyatid rays have been assessed using this approach, yet the application is highly relevant for other rare, vulnerable or endangered species where optimal sample sizes may not be possible. The characterisation of ray diets was assessed through stomach content analysis from 170 individuals of five species. Five broad taxonomic prey categories were common to all species of ray; however, *H. uarnak* is shown to be a crustacean specialist while the remaining four species showed high levels of overlap within their diets. Assessment of the physical impacts related to stingray foraging within an intertidal embayment, previously identified as an area of intense feeding by rays, demonstrated high levels of sediment excavation. As a direct result of bioturbation by stingrays over 21 days, 760 kg of sediment was excavated from an experimental area of 1,500 m^2. Predation effects by rays were examined by experimentally manipulating densities in fixed areas to prevent feeding. Results indicated that some, but not all prey-taxa differed significantly in abundance between treatment and controls. Sampling also allowed a quantitative assessment of infaunal taxa common within the Marine Park, and the potential importance as a prey source for rays, as well as other epibenthic predators. Throughout the course of this study, a new species locality record and parasite-host relationships was described for the parasitic leech *Pterobdella amara* and *Himantura leoparda* and *Urogymnus asperrimus*. This is the first time this leech has been encountered in Western Australia and in combination with a significant gnathiid isopod

larvae infestation; the effects on an individual stingray are documented. A methods paper is also included in this thesis detailing a cost-effective method of tag attachment and retrieval for short-term tracking in reef associated elasmobranchs. Field-testing of galvanic timed releases and the practical application in tagging two individual sharks (*Carcharhinus melanopterus*) and three large stingrays (*Pastinachus atrus* n = 2 and *Urogymnus asperrimus* n = 1) are discussed. Preliminary results of these short-term tracks demonstrated that these methods are a rapid and effective means of tagging elasmobranchs with limited impact on the animal's welfare. This research is the first of its kind at Ningaloo Reef and details critical functional processes and highlights the ecological significance of rays within coral reef environments. It also details current methodologies and techniques trialled for the first time within the context of ecological studies on tropical elasmobranchs. Data presented here can be used to develop or contribute to, conservation and management strategies for this overlooked, yet vulnerable group of fishes.

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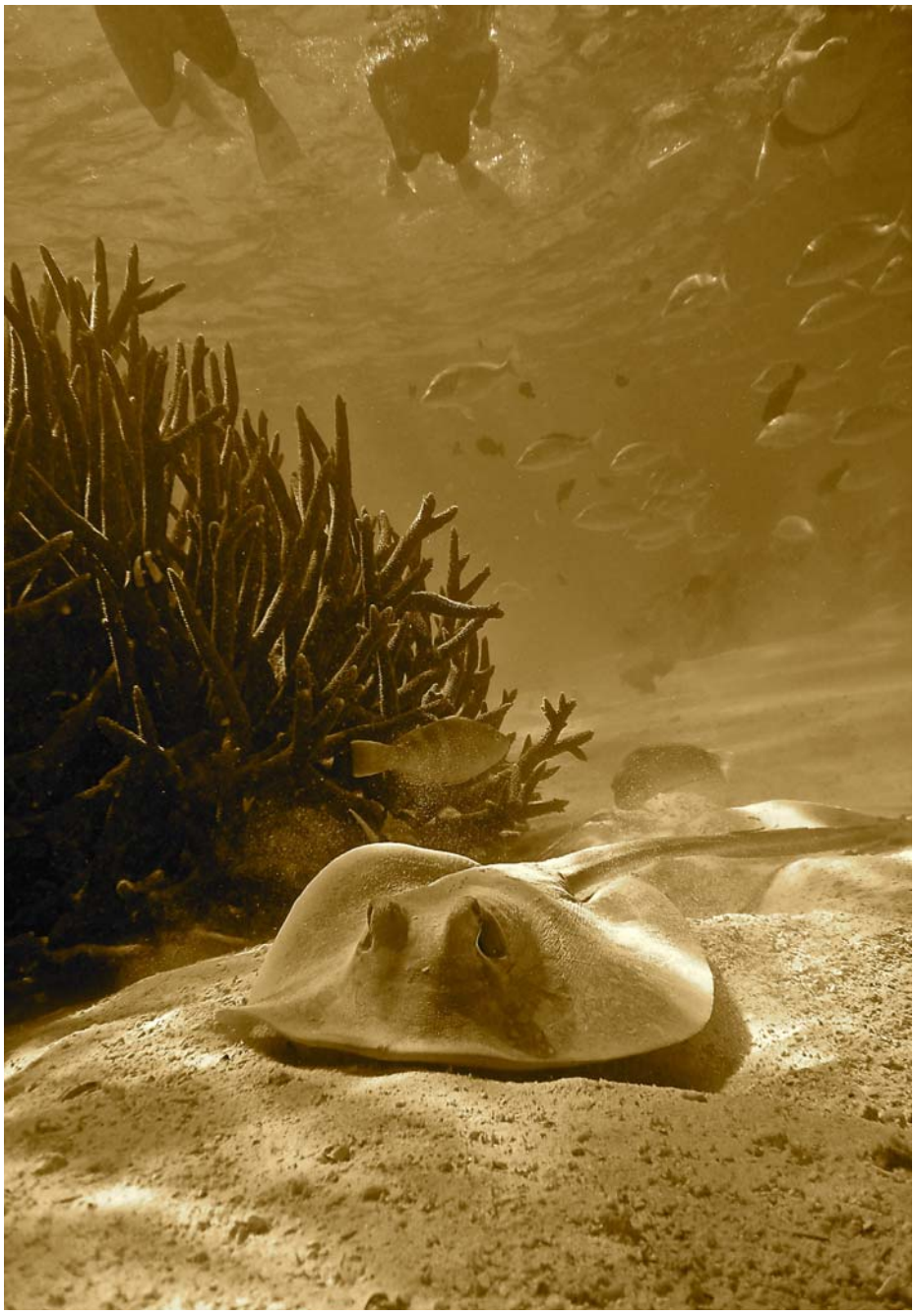
‘*An understanding of the natural world and what's in it, is a source of not only a great curiosity but great fulfilment*’

David Attenborough



Chapter 1:

General Introduction

Classification, diversity and role of rays in the environment

Sharks, rays and chimeras are cartilaginous fishes that are represented by 1,200 extant species and are known collectively as the chondrichthyans (Last & Stevens 2009). The earliest evidence of these fishes appears during the late Silurian period, more than 400 million years ago (Last & Stevens 2009). Sharks, rays and skates are included in the subclass Elasmobranchii (meaning 'strap gills'), while the chimaeras are in the subclass Holocephalii (meaning 'complete head'). Among elasmobranchs, rays are the most abundant and diverse group of fishes, encompassing over 600 living species that have evolved to occupy a broad range of habitats from the Arctic Circle to the tropics, including pelagic, coastal, abyssal and freshwater ecosystems (Last & Stevens 2009). The smallest ray has ~ 20 cm disc width (W_D), while the giant manta ray, *Manta birostris* has been reported to reach over 900 cm W_D, although specimens of 400 cm W_D are common (Last & Stevens 2009).

The most diverse group of rays (superorder) is the Batoidea, which encompasses six orders, 20 families and at least 513 species (McEachran & Dunn 1998; McEachran & Fechhelm 1998; Frisk 2010). Due to their demersal lifestyles, many batoids are cryptobenthic and a bias in research effort towards sharks has led to many species being overlooked and relatively understudied. A recent and comprehensive DNA sequencing study by Naylor and colleagues (2012), suggested a significant amount of previously unreported genetic diversity within the elasmobranchs, including a further 41 species of batoid. The implications of such a discovery validates the necessity

for further research on these under-studied fishes and this thesis targets this knowledge gap. Although rays occur in such a broad range of ecosystems, they are most commonly found in shallow coastal and shelf regions (McEachran & Fechhelm 1998; McEachran & Aschliman 2004; Frisk 2010) where they make up a significant portion of the fish community biomass. In these environments, rays typically occur in a variety of soft-sediment habitats ranging from mangroves and sandy shores to coral reef lagoons (Snelson Jr *et al.* 1988; Gilliam & Sullivan 1993; Cartamil *et al.* 2003). Due to their foraging, rays are agents of disturbance within these ecosystems, facilitating physical, biological and chemical changes in soft sediment habitats, some of which are critical in shaping, modifying and determining habitats (O'Shea *et al.* 2012).

Marine soft sediment environments are the largest yet least known habitats on this planet (Langlois 2005) and it has been estimated that globally, the total volume of all bioturbated sediments is > 20,700 km³ (Teal *et al.* 2008). The scale and rate of bioturbation, like any physical disturbance, is related to the intensity across spatial and temporal scales (Thistle 1981); so given the abundance of rays in coastal soft-sediment ecosystems, their contribution as agents of disturbance is likely to be great, both physically and biologically.

Rays are mesopredators and as such provide critical links between higher and lower trophic levels, exerting top-down control that regulates the structure and dynamics of invertebrate and benthic communities (Wetherbee & Cortés 2004; Navia *et al.* 2007). Furthermore, this intermediary position within marine food webs has been shown to

mediate changes brought about by apex predator loss (Ripple & Beschta 2006; Terborgh *et al.* 2010; Vaudo & Heithaus 2011); therefore understanding the trophic role and impact of rays as mesopredators is critical in elucidating effects of predation in these ecosystems (Vaudo & Heithaus 2011). There are several reports detailing the ecological impacts of feeding by rays; most notably Smith and Merriner (1985), who described the damage to oyster farms and eelgrass beds caused by the feeding of cownose rays *Rhinoptera bonasus* in Chesapeake Bay. Similarly, the spotted eagle ray *Aetobatus narinari* and southern stingray *Dasyatis americana* have been implicated in severe damage to turtlegrass beds as a result of their foraging (Ogden 1980; Zieman 1982; Valentine *et al.* 1994; Nelson 2009). Given the likely importance of rays as predators and agents of disturbance in shallow, coastal environments it is now essential to describe the key aspects of their life history.

Threats, impacts and conservation

Most elasmobranchs have conservative life history traits (slow growth, low reproductive potential and high ages at maturity). This can lead to slow recovery when populations are reduced in size and thus low resilience to fishing mortality (Robbins *et al.* 2006). While the population decline of large sharks has been well documented (e.g. Baum *et al.* 2003, Baum *et al.* 2005, Robbins *et al.* 2006, Myers *et al.* 2007, Heithaus *et al.* 2008, Stevens *et al.* 2009, Ferretti *et al.* 2010), fewer data are available for rays, particularly tropical species. Casey & Myers (1998) discussed bycatch rates of the barndoor skate *Raja laevis* from the northwest Atlantic over almost 50 years and found a sharp decline in biomass, with none having been caught over a 20 year period. Inversely, biomass of two similar yet smaller species, the smooth skate *Raja senta* and thorny skate *Amblyraja radiata*, was shown to

increase over the same time period. This study recommended a more vigilant examination of historical data to identify such extinction threats before it is too late, particularly for smaller, less conspicuous species.

Dasyatid rays make up a significant portion of artisanal chondrichthyan fisheries, particularly in Mexico (Bonfil 1994; Vannuccini 1999; Smith *et al.* 2008), Singapore, Thailand, Malaysia (Last & Compagno 1999) and Indonesia. Indonesia has reportedly the largest fishery in the world, landing 105,000 and 118,000 tonnes in 2002 and 2003 respectively (White *et al.* 2006). More recently, rays in the Mediterranean Sea have suffered from significant declines in abundance, diversity and range, particularly the pelagic stingray *Pteroplatytrygon violacea*, which is a significant component of long-line bycatch (Piovano *et al.* 2010) as well as being common bycatch throughout the Pacific (Mollet 2002). In Australia many species are vulnerable to benthic trawl fisheries (Stobutzki *et al.* 2002), but a move toward including bycatch species within ecosystem-based fisheries management has resulted in an interest in stingray conservation (Pierce & Bennett 2010).

Although the future of ray populations inhabiting shallow coastal waters remains unclear (Heithaus *et al.* 2010), fishing pressure is undoubtedly a major concern for all populations of coastal elasmobranchs with sharks perhaps facing the greatest challenges for conservation and management due to increasing demand for fins and meat (Heithaus *et al.* 2010). Unfortunately, fishing is not the only threat to elasmobranchs as coastal marine ecosystems by their very nature are more likely to become impacted by human pressures due to their close proximity to large human populations. Impacts include pollution (Froese & Garilao 2002), habitat loss or

degradation from anthropogenic stresses (Worm *et al*. 2006) and general changes brought about by climate shifts, such as sea-level rise (Field *et al.* 2009), increased ocean acidification (Orr *et al.* 2005), increased minimum and maximum temperatures (Vose *et al.* 2005) and changes in salinity (Clarke *et al.* 2002).

The reliance on certain habitats by species, or habitat specificity also needs to be examined when implementing management and/or conservation solutions. Attachment to specific habitats may be important because even isolated and remote populations can be vulnerable to habitat degradation (Camhi *et al.* 2009). Coastal populations however, are more vulnerable and habitat degradation may influence choice of habitat use or selection by some species; for example, some coastal shark species may use certain cues for habitat selection (Heithaus *et al.* 2002, 2006; Speed *et al.* 2010), e.g. prey availability or presence/absence of con-specifics. If biotic or abiotic cues are responsible for habitat selectivity, then degradation in some coastal habitats may result in population declines or in extreme circumstances, localised extinctions.

Demography

Sampling populations of coastal rays is often very difficult making accurate estimations of population size and demographic patterns problematic. For this reason, very little, if any, comprehensive demographic data of coastal ray populations exists for either temperate or tropical ecosystems, and accordingly, most data are derived from fishery catch rates or anecdotal reports. Like all elasmobranch species, the main problem lies in deriving fishery-independent estimates of species abundance (Simpfendorfer 2005). For rays, this is typically correlated to gear type,

for example, rays are hard to enmesh in gill nets and due to their relatively low movement rates, static sampling is difficult (White & Potter 2004, Pierce *et al.* 2011). Typical faunal surveys are also problematic because many rays are often buried or can be cryptic, making them difficult to detect, particularly in larger areas where techniques such as manta tows are essential.

Catch rates of rays in commercial fisheries are an important tool for assessing population structure and for demographic analysis; however, there are inherent problems with using such data. Rays caught as bycatch may be discarded without being reported (Stobutzki *et al.* 2002) or landing sites in remote areas may be inaccessible to survey (White & Dharmadi 2007), which contribute to the paucity of data available on catch rates and the demography of certain species. So little is known about population structures and ecological roles of coastal ray species that new locality records are common and even new species are being found in relatively well-surveyed ecosystems, for example, at Ningaloo Reef a new species of mask ray was recently described - *Neotrygon ningalooensis*; Last, White and Puckride (2010).

The collection of life-history data may go a long way to addressing basic needs for effective management, especially when assessing stocks using fisheries models (Matta & Gunderson 2007) and for vulnerable species with intrinsic susceptibility to population declines. With over 600 living species of ray, there is naturally a large variation in growth and maximum sizes. It is often reported that larger-bodied fishes have greater longevity and attain greater average ages than smaller bodied fishes (Blueweiss *et al.* 1978); however some of the oldest reported elasmobranchs have

been species that attain comparatively smaller adult sizes. For example, the spiny dogfish *Squalas acanthias* can reach 65 – 70 years ((160 cm total length (L_T)) (Ketchen 1975; Hoenig & Gruber 1990); the school shark *Galeorhinus australis* 43 years (193 cm L_T) (Hoenig & Gruber 1990); bat rays *Myliobatis californica* 23 years (180 cm W_D) (Martin & Cailliet 1988, Hoenig & Gruber 1990); while the thorny skate *Amblyraja radiata* was reported by McPhie and Campana (2009) to attain an age of 28 years (105 cm L_T).

Aims

The rapid growth in shark studies over recent years has highlighted specific areas of research that can be applied to rays in a similar context, on account of comparable life histories and potential impacts threatening populations. Until now, the most comprehensive survey of sharks and rays within the Ningaloo Reef Marine Park was conducted by Stevens and colleagues (2009). This study concluded that any results need to be treated as preliminary and that further research was required. The broad objectives of the present study were to address these research needs by providing baseline data on certain biological and ecological functions of five numerically abundant, sympatric species of stingray (Dasyatidae) within the lagoonal habitat of Ningaloo Reef (Figure 1.1). Ningaloo is one of the largest fringing reefs in the world and encompasses a diverse range of habitats including mangroves, rocky shores, sandy shores, coral reefs, vast sand flats and a convoluted, geo-morphologically complex coastline which has created a series of sheltered coves and protected bays. The lagoon at Ningaloo is generally shallow (≤ 10 m) and the reef crest varies in distance from the shore between 100 m and 4 km (Stevens *et al.* 2009). Ningaloo has

very little coastal development with only two main settlements along its 260 km coast, and as such offers a valuable opportunity to research a relatively pristine environment. Both temperate and tropical currents converge at Ningaloo resulting in high biotic diversity (Waayers 2010). Due to the physical nature of the lagoon and shallow intertidal areas, Ningaloo Reef supports one of the largest and most diverse assemblages of elasmobranchs in Australia, including an estimated 59 species of shark and 34 species of ray found in < 200 m (Last & Stevens 2009, Stevens *et al.* 2009).

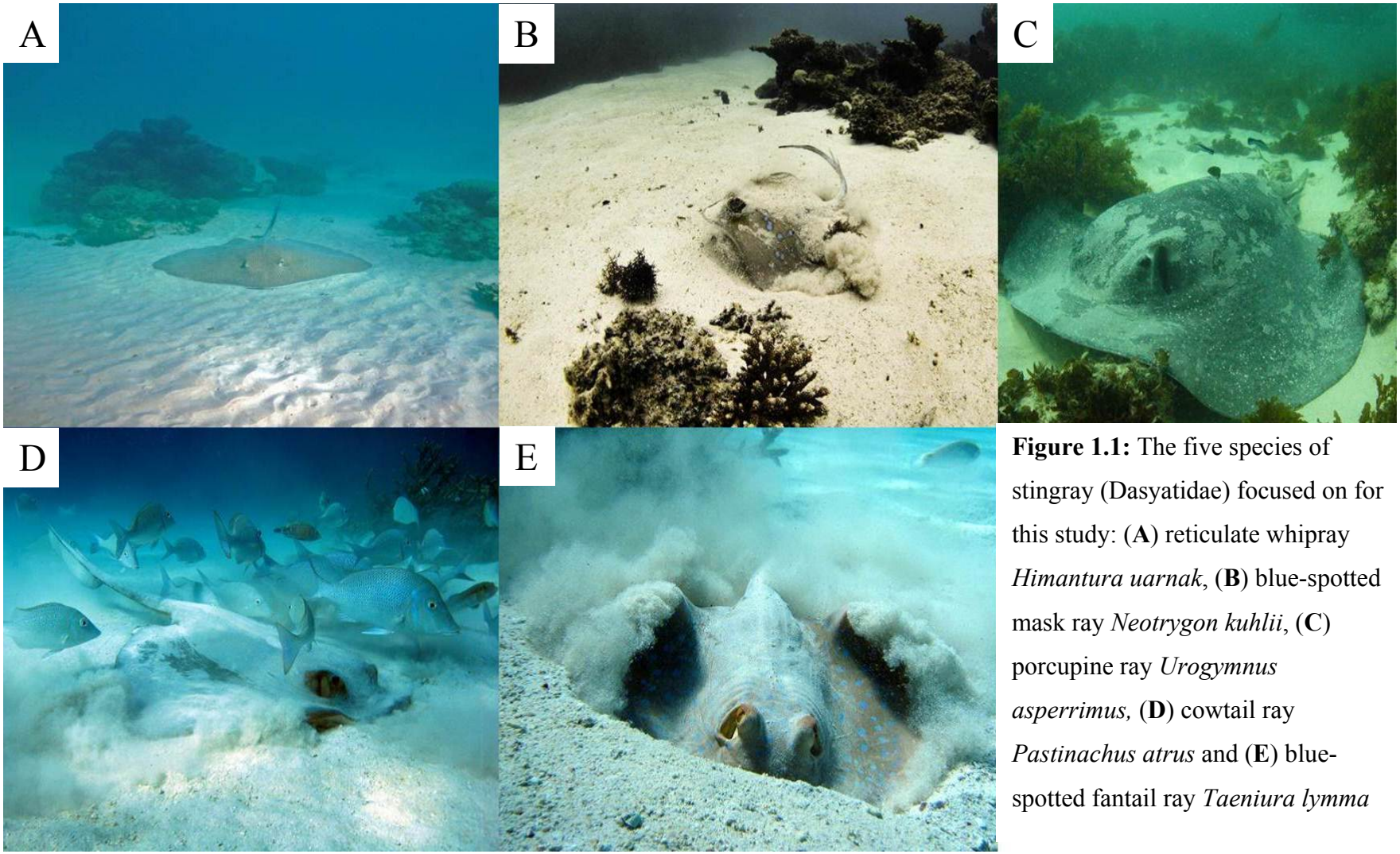


Figure 1.1: The five species of stingray (Dasyatidae) focused on for this study: (**A**) reticulate whipray *Himantura uarnak*, (**B**) blue-spotted mask ray *Neotrygon kuhlii*, (**C**) porcupine ray *Urogymnus asperrimus,* (**D**) cowtail ray *Pastinachus atrus* and (**E**) blue-spotted fantail ray *Taeniura lymma*

With this in mind, the overall aims of this thesis are to examine critical ecological processes and functional biology of five sympatric species of stingray from a range of habitats within the Ningaloo Marine Park as follows:

1. Provide a detailed description of the methods used to catch and sample stingrays for use in scientific studies that conforms to Australian codes of practice (**chapter 2**).
2. Describe the age structure and growth parameters of five species of stingray by using a combination of statistical methods, including a Bayesian framework, to optimise parameter estimates for hard-to-sample species (**chapter 3**).
3. Characterise the diets of five sympatric species of stingray and describe patterns of overlap or partitioning within their diets (**chapter 4**).
4. Quantify the physical effect of bioturbation as a consequence of feeding by rays in an area of high abundance (**chapter 5**).
5. Assess the biological impacts of stingray feeding using an experimental manipulation to exclude predation from occurring in specific sites using cages (**chapter 6**).
6. Write a new locality description for a species of parasitic leech and two new host stingrays encountered during the course of this research (**chapter 7**).
7. Assess methods of acoustic tag attachment and retrieval when monitoring short-term movements in elasmobranchs in a coral reef environment (**chapter 8**).

8. Provide a summary of key findings and broader implications of this research, including future directions that are required to enable further understanding of stingrays in these ecosystems (**chapter 9**).

Thesis structure

Chapter 2 is a technical assessment of methods that were developed as part of this research for the safe and ethical lethal sampling of stingrays. Ethical considerations when lethal sampling of any animal need to be concise, and in Australia a rigorous code of practice exists that requires regular reporting and justification for using wild animals in scientific research. This chapter focuses on safe fieldwork practices when killing stingrays to ensure researcher safety and methods to guarantee minimal pain and suffering to the animals. Pain reception in elasmobranchs is discussed and compared with higher vertebrates.

Chapter 3 is an assessment of the age structure and growth rates of four species of ray common to the marine park. The main focus of this chapter however, is the development of different statistical methods to increase the precision for growth parameters due to small sample sizes. Maximum likelihood is used to compare a range of growth models to determine which model best describes the data. A Bayesian framework is then applied to the model of best fit using Markov Chain Monte Carlo (MCMC) simulations to sample posterior distributions. Difficulties in estimating accurate growth parameters are discussed and the applications for using a Bayesian approach for a range of difficult-to-sample species is included.

Chapter 4 characterises the diets of five sympatric species of stingray through stomach content analysis. The focus of this chapter is to determine broad differences in the dietary content of rays and evaluate whether evidence for partitioning of resources exists, or whether diets overlap. Previous studies describing the dietary preferences of tropical stingrays are limited in sample size or representative species from one area. Hypotheses are proposed relating to partitioning of dietary resources to relax competition pressure between species.

Chapter 5 provides the details of a sampling program that aims to quantify the physical effect of bioturbation by stingrays at an intertidal embayment in the northern section of the Marine Park. The focus of this chapter is to determine sediment excavation rates, feeding pit creation and persistence in the environment. Quantities of sediments in terms of weight and volume are determined, and the probability of pit presence/absence is modelled using GLMM. Rates of bioturbation are compared with published accounts of other taxa and the ecological importance of bioturbation by rays is discussed.

Chapter 6 describes a study where impacts associated with stingray feeding on benthic and infaunal communities were experimentally assessed. Large aggregations of feeding rays were identified from chapter two, providing the opportunity to determine changes in the abundance of invertebrates between caged treatments and un-caged controls. Experimental manipulations are useful tools in ecology, yet few data exist when applied to the effects of feeding in

tropical dasyatids. Implications for management of areas of high ray abundance are discussed, as are suggestions to refine the experiment for future work.

Chapter 7 is the brief description of a new locality record for a species of parasitic leech and two new host stingray descriptions that were opportunistically encountered during this project.

Chapter 8 provides an assessment of methods used to tag and track reef sharks and stingrays with acoustic transmitters in a coral reef environment. The main focus for this chapter was for short-term tracking i.e. 24 – 48 hours and the different methods for tag attachment in sharks and rays. Static field-testing of galvanised timed releases are discussed and preliminary results from five individual tracks (two sharks and three stingrays) are presented. With a rapid increase in telemetry studies, this chapter provides a relevant insight into cost effective methods for determining fine scale movements for reef-associated elasmobranchs.

Chapter 9 is a general discussion, detailing the key findings of this study, while identifying future directions for research.

Chapter 2:

Lethal Sampling of Stingrays for Research

Introduction

Ecological research often involves the collection of certain life history data that requires invasive and sometimes destructive sampling methods. Although lethal collection may come at a cost to a population, such sampling provides essential data that cannot be obtained in any other way (Heupel & Simpfendorfer 2010). Examples of this type of research include age and growth assessment in elasmobranchs, which requires the removal of vertebral sections to analyse banded calcium deposits within the centra, reproductive studies that typically involve macroscopic inspection of internal organs, combined with tissue harvest for histological analysis and some dietary studies that require dissection of the gut for analysis of contents. Alternatives to lethal sampling in elasmobranchs do exist, such as stomach lavaging (Barnett *et al.* 2010) or stable isotope analysis (Speed *et al.* 2011) instead of stomach dissection, the use of caudal thorns (where present) for ageing instead of vertebral extraction (Matta & Gunderson 2007) and ultrasonography for the assessment of maturity in oviparous species (Whittamore *et al.* 2010). While these methods are valid, ethical and welfare-based, they are not always appropriate or even possible for some species. This leaves researchers little choice but to use lethal sampling for collection of some ecological data.

In tropical Australia, stingrays (Dasyatidae) have been traditionally obtained from the bycatch of commercial fishers, primarily from the Northern Prawn Trawl Fishery. However, since the early 2000's, the use of bycatch reduction and turtle

exclusion devices (BRDs & TEDs) has been mandatory, significantly reducing the incidental capture of elasmobranchs (Brewer *et al.* 2006). In order for certain research on rays to continue, alternative collection methods must be adopted. Given the size stingrays can attain (> 200 cm disc width W_D) and the potential for harm associated with the large and toxic barbs present on their tails, such collections must be undertaken with great care to maintain a safe environment for researchers. Equal consideration must also be given to the ethical treatment of the target animals and minimisation of the pain and suffering that they may experience during collection.

The overall objective of this study was to conduct a lethal sampling program in order to collect a large suite of demographic (age, growth and population structure) and ecological (diet, reproduction, genetics) data on stingrays in a coral reef environment. Here, I detail methods of my lethal sampling program used to collect 170 individual stingrays at Ningaloo Reef, Western Australia while ensuring both animal welfare and the safety of researchers.

Methods

Study location and species

This study was part of a collaborative research effort assessing the ecological role of stingrays within the Ningaloo Reef Marine Park (NRMP) in Western Australia (Figure 2.1). The NRMP is the largest fringing reef in Australia and has a geo-morphologically complex coastline, creating a diverse range of habitats, supporting a high diversity of flora and fauna, particularly elasmobranchs (Last & Stevens 2009).

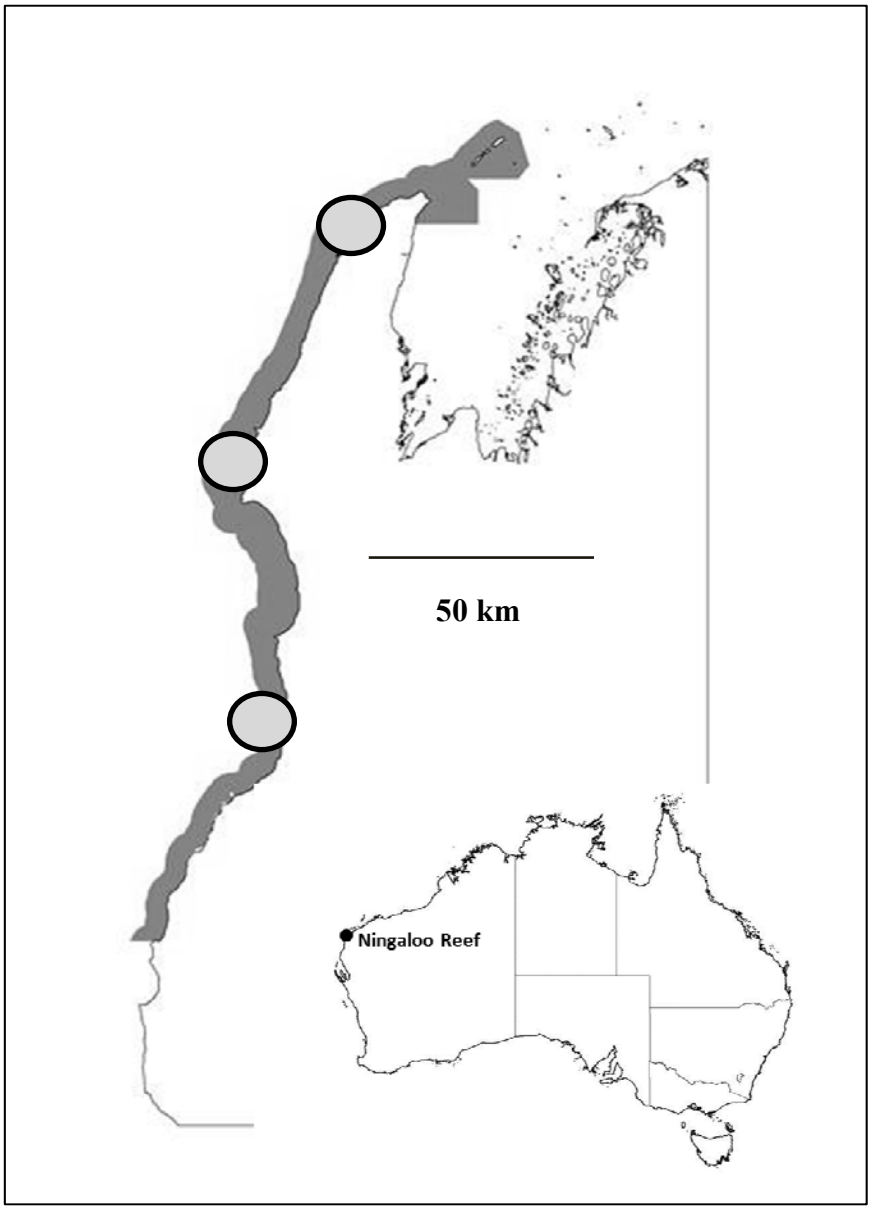


Figure 2.1: Map of the Ningaloo Reef Marine Park, highlighting the three broad areas targeted for sampling

Sampling took place at 18 locations within the Marine Park (Table 2.1) between November 2009 and November 2011. I sampled 170 rays of five sympatric species including the blue-spotted mask *Neotrygon kuhlii* (Müller & Henle 1841) (n = 36), cowtail *Pastinachus atrus* (Macleay 1883) (n = 43), blue-spotted fantail

Taeniura lymma (Forsskål 1775), (n = 54) porcupine *Urogymnus asperrimus*, (Bloch & Schneider 1801), (n = 13) rays and the reticulate whipray *Himantura uarnak* (Forsskål 1775), (n = 24) (Table 2.2).

Table 2.1: Sampling sites and associated waypoints within the Ningaloo Reef Marine Park

Site Name	Latitude, Longitude
Tantabiddi South	-21.9386, 113.9664
Mangrove Bay	-21.9762, 113.9598
Ranger Bay	-21.9403, 113.9674
Ranger Bay back reef	-21.9397, 113.9707
Point Look	-21.9968, 113.9252
Point Look back reef	-21.9987, 113.9140
Winderabandi Point	-22.4960, 113.7042
North Lefroy Bay	-22.5155, 113.7070
South Lefroy Bay	-22.5337, 113.6791
Point Edgar	-22.5803, 113.6519
Stanley Pool	-22.9455, 113.7857
Point Maud	-23.1184, 113.7630
The Maze	-23.1218, 113.7514
Skeleton Bay north	-23.1255, 113.7694
Skeleton Bay south	-23.1335, 113.7703
Five Fingers Reef	-23.1748, 113.7592
Monk's Head	-23.2135, 113.7651
South Passage Coral Bay	-23.2204, 113.7695

Sampling Design

All rays were captured and euthanased *in situ*, from inshore and offshore sites. Inshore sites were accessed from beaches and typically consisted of water ≤ 2 m deep. Offshore sites were always within the lagoon and accessed from a research vessel. Maximum water depth never exceeded 8 m. Of the five species

sampled, two were smaller (*T. lymma* and *N. kuhlii*), reaching maximum sizes of 35 cm and 47 cm W_D respectively (Last & Stevens 2009), while three species attain much larger sizes (*Pastinachus atrus*, 200 cm W_D; *Himanutra uarnak,* 140 cm and *Urogymnus asperrimus*, 115 cm W_D) (Last & Stevens 2009). Separate sampling methods were designed based on size and sites of capture. At inshore sites, rays were generally smaller (≤ 100 cm W_D), so beach seines and hand nets were used for capture. Large rays (≥ 100 cm W_D) and those caught offshore were euthanased with spear guns (Undersee woodie MKII 1700, MKII 1400 and Mares Cyrano 700) while free diving.

Table 2.2: Morphometric and demographic data of rays sampled

	H. uarnak			*N. kuhlii*			*P. atrus*			*T. lymma*			*U. asperrimus*		
	W_D (cm)	**Body mass (kg)**	**n**	**W_D (cm)**	**Body mass (kg)**	**n**	**W_D (cm)**	**Body mass (kg)**	**n**	**W_D (cm)**	**Body mass (kg)**	**n**	**W_D (cm)**	**Body mass (kg)**	**n**
Male	107.3 ± 35.9	40.1 ± 25.2	14	33.8 ±10.6	1.8 ± 0.6	36	69.2 ± 39.6	15.9 ± 29.8	43	25.7 ± 4.2	1.1 ± 0.68	54	107.1 ± 8.2	64.7 ±16.8	13
Female	87.7 ± 35.9	21.1 ± 23.4	10	33.5 ± 9.8	1.8 ± 0.8	25	63.2 ± 31.5	11.9 ± 20.7	21	24.5 ± 3.8	1.5 ± 2.2	29	100.7 ± 5.2	54.3 ±11.6	8
Juvenile	48.1 ± 9.9	3.3 ± 3.2	8	33.5 ±12.1	1.8 ± 0.6	11	71.5 ± 43.6	20.2± 36.5	22	26.9 ± 4.2	1.3 ± 0.8	25	0	0	0
Adult	115.1 ± 26.5	44.1 ± 20.8	16	15.2 ± 2.1	0.7 ± 0.61	9	45.8 ± 12.6	2.7 ± 2.8	28	0.2 ± 0.2	16.5 ± 2.5	9	115.0 ± 5.1	081.2 ± 7.2	5
All	101.4 ± 38.2	32.2 ± 25.8	24	38.8 ± 3.3	1.9 ± 0.8	27	117.6 ± 31.3	49.6 ± 47.4	15	26.2 ± 3.6	1.5 ±1.6	45	107.1 ± 8.2	64.7 ± 16.8	13
Range	37—145.5	1—68.5	24	12.5—47	0.8—3.7	27	27—177	0.7—136.4	15	14—32.5	0.8—3.2	45	93—118.5	38.5—90.4	13

Considerations

The rays used as part of this research were selected because they are numerically abundant within the NRMP (Last & Stevens 2009) and have an important ecological role and impact within these environments. It was also considered essential to spread my sampling effort among 18 sites within 3 broader locations – northern, mid and southern sections of the Marine Park to minimise impact on any single population. In order to ensure personal safety, teams consisted of two free divers, with a spotter on a research vessel boat close by and a skipper. The first diver would descend to the ray and position themselves before firing the spear. The second diver at the surface would observe and if required also free-dive to take a second shot or assist the first diver if any issues arose. Ten metre float lines were attached to the loading butt of each speargun so after the spear was fired, the diver could simply let go of the speargun to avoid potential entanglement or dislodgment of the spear if the animal bolted. Surface floats also allowed divers to return to the boat without losing sight of the ray. Rays were then lifted to the boat using the float lines before the tail was secured to avoid injury, either accidentally or through a post-mortem muscle spasm. Once the tail was secured, the spears were removed and for very large rays, a rope was passed through the spiracles in order to lift it into the boat.

Lethal sampling techniques

The cartilaginous brain casings of rays are penetrated easily by spears at close range ensuring a fast death with minimal suffering. This was deemed the most efficient, direct and safest way to euthanase rays while operating in deeper water. For smaller rays at inshore sites, one ray at a time was caught by actively herding them into the

net, rather than passive trapping. Once caught, they were bought into the shallows in a hand net and killed by directly destroying the brain and/or severing the spinal cord immediately behind the head. This was done using a reinforced steel commercial diver's knife. An assessment of corneal reflex was used to confirm death, which involved touching the eye, which would retract if brain function still existed.

Rays are relatively sedentary animals and during offshore sampling were encountered either feeding or resting. This enabled divers to get close enough to allow very accurate shots when firing spears. The three species of larger ray all exhibited different behaviours when first encountered. *Himantura uarnak* were generally buried and inactive in soft sediments within close proximity of the coral reef, seemingly favouring an edge habitat. This species made no attempt to evade the boat or divers. *Urogymnus asperrimus* was detectable due to the large sediment plumes arising from vigorous feeding that was typical of this species when encountered. They also made no effort to move and for this reason, these two species were generally killed instantly with little or no suffering (as perceived by the divers). The third species, *Pastinachus atrus* tended to act more unpredictably and being the largest species of the five, was treated with more caution. Individual *P. atrus* were either feeding or resting in sandy patches within the lagoonal reef complex and would generally flee when the boat approached.

Due to excellent camouflage they are harder to see than other large species however, evasive behaviour in response to the boat's presence would expose previously unnoticed rays. Avoidance behaviour exhibited by this species consisted of

individuals swimming to nearby coral heads or fragmented reef structure and burying themselves in the sand immediately adjacent to the reef. They are very large and conspicuous animals when swimming and because of the good water clarity at Ningaloo most of the year, they were easily followed, precluding the need to chase them in a way that might have caused undue stress.

Once located, unambiguous identification of the ray was made from the surface to ensure non-targeted species were not collected. Divers entered the water up current from the ray and approached slowly. It was essential to not alarm the rays so they did not react defensively or initiate a flight response. Rays were approached from the front at an angle of not less than 45º from a line running in the direction in which the ray was facing (Figure 2.2). This ensured the ray was aware of diver's presence as well as having a space away from the diver in which to flee so it was not 'cornered'. This also enabled divers to return to the surface to observe at a safe distance from the dying, or fleeing animal. In every case, rays (whether speared or not) rapidly swam away from the diver across the benthos.

Observations were made after the spear had been fired to ensure the animal was killed outright rather than seriously wounded. The spearguns used were 1.7 and 1.4 m in length, and because they were wooden, remained slightly positively buoyant, which allowed the gun to be extended away from the diver's body, thereby increasing the distance from the diver to the target ray, while still enabling firing at near point blank range. The target area on the ray was the interorbital space and when hit, enabled the spear to penetrate the brain resulting in an immediate death.

Post-mortem movements, including beating of the pectoral fins and some erratic swimming were common among all rays for up to several minutes after death. A small number of rays (predominantly *U. asperrimus*) did not move at all after the spear was fired. Several individual *P. atrus* bolted prior to the spear being fired and this was always away from divers and the perceived threat. In these instances, spears were not fired, but rays were easily tracked from above the surface. On 11 occasions rays did flee at the same time a spear was fired, which either resulted in a miss, the spear glancing off the ray, or striking the ray with a non-lethal shot. When the latter occurred, divers returned immediately to the boat, where the ray was tracked from the surface and when it came to rest, a second spear was fired. Every ray from the two smaller species was killed with one direct hit.

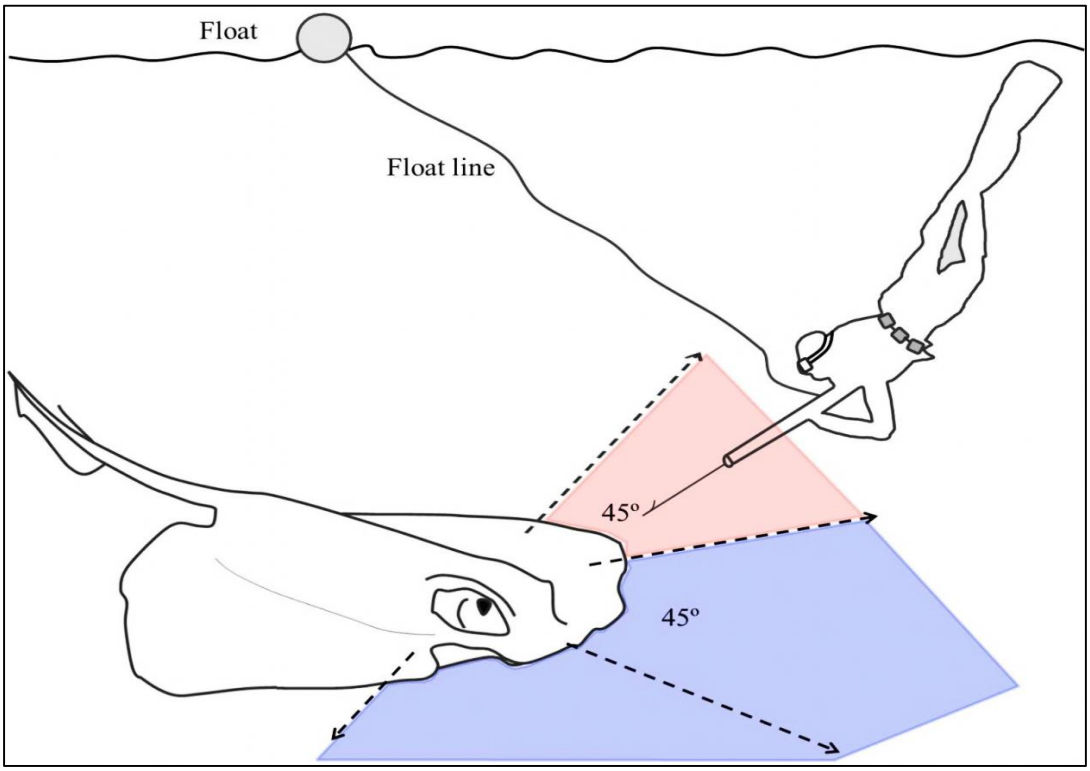


Figure 2.2: Optimum approach by a diver to a ray while free diving. The arrows represent the directions in which rays will typically swim based on behaviours observed during this research. The blue shaded area highlights the ideal angle of flight, while the red poses the greatest risk. It is important to note, that both red are blue areas are not mutually exclusive, and whichever angle the diver approaches from, should always be considered 'red'

Discussion

The use of lethal sampling in ecological research is sometimes unavoidable; however, the development of safe and ethical techniques for procuring data remains at the core of any such sampling program. The methods outlined here were developed to acquire data via lethal sampling, while maintaining the highest safety

standards and ensuring ethical standards were also met. The use of spearguns to kill rays was effective when compared to other methods. For example, static sampling using hook and line can be non-selective, time consuming and stressful for any animal caught. Of the 170 rays euthanased for this research, 94% (n = 159) were killed within an estimated 10 — 30 seconds by spearing or insertion of a knife into the brain. Of the remaining 6% (n = 11), complications arose when the ray evaded the diver at the moment of firing the speargun, resulting in a non-lethal strike. While the protocol described in this paper was designed to prevent this situation and was largely successful, I failed to kill the target animal outright in 11 cases. However, the use of float lines allowed immediate tracking of the ray and administration of a subsequent lethal shot. In each of these cases, the maximum time between the first and second shot was no longer than approximately five — eight minutes. Throughout the course of this 24-month program, no incidents were reported or injuries sustained to any member of the research team.

Pain perception in elasmobranchs

Most of our knowledge of pain perception in animals comes from higher vertebrates, such as mammals and birds (Braithwaite & Boulcott 2007). This subject is a relatively new area of study for fish and has resulted in conflicting views (Sneddon 2003) but see (Sneddon *et al.* 2003a). Nociceptors are part of the neural apparatus associated with pain perception in most animals, including fish. These are capable of detecting noxious or potentially damaging stimuli (Braithwaite *&* Boulcott 2007). Nociceptive pathways comprise either A-delta nerve fibres or C fibres with the latter

being the predominant nerve fibre type in higher vertebrates involved in pain perception (Sneddon *et al.* 2003a). Some of the few studies conducted on elasmobranchs found the presence of A-delta fibres in some species (Atlantic stingray, *Dasyatis sabina* and the pink whipray*, Himantura fai*), but C fibres were either absent or found in very low numbers suggesting pain perception may be reduced, when compared to those species where C fibres are present (Coggeshall *et al.* 1978, Leonard 1985, Snow *et al.* 1993, Snow *et al.* 1996, Braithwaite & Boulcott 2007). This also appears to be the case for the spotted eagle ray, *Aetobatus narinari* (n = 1), cownose ray, *Rhinoptera bonasus* (n = 2) (Coggeshall *et al.* 1978), black tip reef shark, *Carcharhinus melanopterus* (n = 3), shovelnose ray, (*Glaucostegus typus*) (n = 4) and a member of the whipray genus, *Himantura* sp. (n = 3) (Snow *et al.* 1993). Given that some authors have concluded that C fibres are essential for the sensation of pain, it may well be that pain perception in those species that lack them might have little relevance to survival.

Outcomes

The lethal sampling undertaken in my research program has led to a better understanding of the key ecological functions of stingrays in tropical reef environments and can be used to formulate better management and conservation strategies. While my study is the first comprehensive assessment of the ecology and biology of stingrays within the NRMP, it has implications in a broader Indo-Pacific context where rays are harvested for meat, leather and gill filaments (White *et al.* 2006). My lethal sampling allowed the evaluation of dietary preferences, contrasted

the feeding habits of five sympatric species of ray and assessed the potential for resource partitioning, which can be used to further understand the importance of these species as mesopredators (O'shea *et al.* accepted). Assessment of the age and growth of these rays allows insights into population structure and biological traits of rays within the NRMP (O'shea *et al.* in review). Further work has described the use of DNA barcoding as a tool for identifying cryptic species, aiding field identification and highlighting species complexes (Cerutti-Pereyra *et al.* 2012).

Macroscopic analyses of reproductive organs in combination with age and growth data have allowed information about size at maturity to be introduced into fisheries management strategies (O'Shea *et al.* in preparation). An unexpected outcome from this research was the description of a new locality record for a parasitic leech and two new host relationships, not previously recorded along the west coast of Australia (O'Shea 2010). Finally, collection of these rays has allowed a study of the ecological and phylogenetic factors influencing the distribution and number of electroreceptor sensory organs (Kempster *et al.* 2011). There are six further research papers detailing vision, electro-sensory morphology, neurone populations and cranial nerve counts that are currently in preparation, directly resulting from this lethal sampling program.

Conclusions

Using the methods described here I have successfully completed a lethal sampling program for dasyatid rays. My approach factored in ethical considerations, researcher safety and the potential for quality data to be collected. Most animals

targeted as part of this research were euthanased quickly, efficiently and potentially without experiencing pain. While the notion of lethal sampling for research will continue to be the subject of debate among all sections of the community, it is hoped that the careful and complete analysis of the samples collected by my study will remove the need for the collection of such types of data in the future.

Chapter 3:

Optimising growth parameter estimates in tropical stingrays with low sample sizes

Introduction

Elasmobranchs face increasing fishing pressure on a global scale due to a combination of rising consumer demand and life history characteristics that make them extremely vulnerable to over-harvest (Hoenig & Gruber 1990; Dulvy & Forrest 2010). In Australian waters, batoids have been largely overlooked by researchers and managers involved in commercial fisheries, primarily due to their low commercial value in comparison to sharks. However, they are still significant components of bycatch, particularly in penaeid fisheries (Dell *et al.* 2009). In a wider Indo-Pacific context, many demersal rays such as dasyatids are targeted in artisanal and small-scale fisheries for their meat and leather (White *et al.* 2006) yet for the most part, there is a lack of even the most basic life-history data for such species. Given that knowledge of growth rates and age structure is essential for any assessment of the ability of populations to sustain and recover from harvest, studies on the age and growth of batoids are urgently required.

Chondrichthyan fishes have been aged by counting growth band pairs in vertebral sections for over 90 years (Ridewood 1921). While such techniques are reliable, accurate and common-place, the acquisition of adequate sample sizes remains a major challenge for age and growth studies (Cailliet & Goldman 2004), particularly for those species that are poorly represented in commercial fisheries (the most

common method for sourcing specimens) (Davenport & Stevens 1988) due to gear selectivity (Thorson & Simpfendorfer 2009; Smart *et al.* 2012) and/or spatially restricted sampling (Simpfendorfer 2005). Low sample sizes hinder age and growth research because they present challenges to classical approaches to the statistical estimation of growth parameters. For example, low sample sizes may result in techniques such as nonlinear regression providing estimates that are not an accurate reflection of growth patterns (Siegfried & Sansó 2006). However, Bayesian statistics provide one means of overcoming these issues (Siegfried & Sansó 2006) because they can increase the precision of growth parameter estimates through the use of informative prior data.

Here, I take a synthetic approach to the development of robust estimates of growth parameters of four sympatric species of dasyatid ray at Ningaloo Reef, Western Australia. I combined different analytical methods, including Bayesian models, to improve growth parameter estimation for these species where only very limited sample sizes were available. I determined the best growth model using a likelihood framework and approximated the posterior distribution of growth parameters using literature-derived priors within a Bayesian framework. This approach has relevance to any marine species where sample sizes are restricted, particularly in species-diverse ecosystems such as coral reefs.

Methods

Study sites and sample collection

A total of 170 rays (*H. uarnak* Forsskål 1775, n = 24; *N. kuhlii* Müller & Henle, 1841, n = 36; *P. atrus* Macleay 1883, n = 43; *T. lymma* Forsskål 1775, n = 54 and *U. asperrimus* Bloch & Schneider 1801, n = 13) were collected for age analysis between February 2010 and February 2011 in the shallow (≤ 10 m water depth) lagoon of the Ningaloo Reef Marine Park (Figure 3.1). Due to logistical constraints, sampling was restricted to the months of February (38%) and August and September (62%). Small rays were caught in hand nets and larger individuals using spear guns following methods outlined in chapter 2. Logistic, environmental and ethical constraints resulted in small sample sizes, in contrast to other studies that have been able to use large seine nets over sand flats or sourced rays from commercial fishers (e.g. Jacobsen & Bennett 2011). This was not possible at Ningaloo Reef where the lagoon and nearshore intertidal areas are dominated by coral reef and commercial fishing activities are not permitted within the Marine Park.

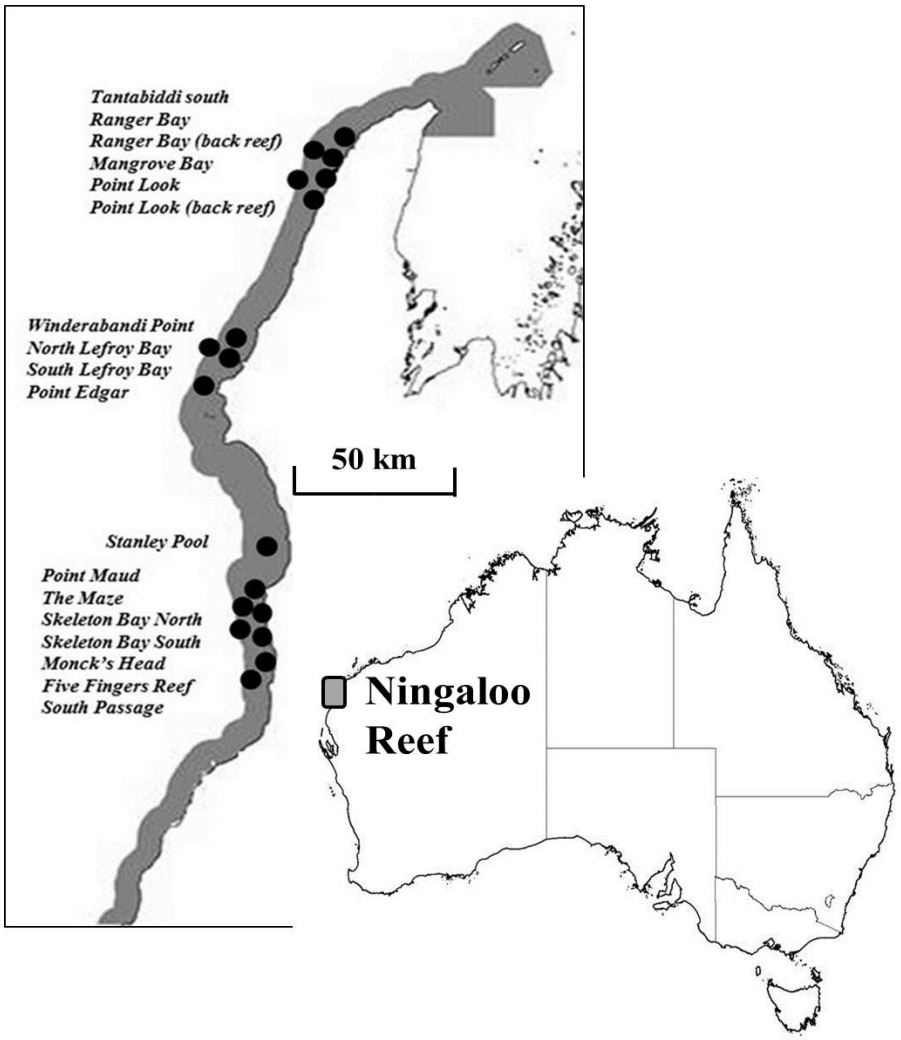


Figure 3.1: Map of the eighteen study sites within the Ningaloo Reef Marine Park, Western Australia

Age validation

Fifty-two rays (*H. uarnak* n = 8; *N. kuhlii* n = 11; *P. atrus* n = 19 and *T. lymma,* n = 14) were caught at Skeleton (n = 30; 23° 8.378'S 113° 46.240'E) and Mangrove (n = 22; 21°58.385' S 113°56.99' E) Bays in the Ningaloo Marine Park between November 2009 and November 2010. Each ray was weighed, its disc width (W_D) and

total length (L_T) were measured and the animal was fitted with a T-bar spaghetti tag. Rays were then injected with calcein at 3 ml/kg of body weight and then released.

Vertebrae preparation

Vertebrae were removed from the rays collected for age analysis, posterior to the cranium at the widest point of the animal and stored in a freezer within 8 hours of excision for transport to the laboratory. In the lab, centra were cleaned of connective tissue before being placed in a 5% sodium hypochlorite solution for between 30 minutes and 2 hours depending on their size. The samples were then soaked in distilled water for ten minutes before being air-dried overnight. Next, three centra were embedded in clear polyester casting resin and left to set overnight, after which 350 µm sections were cut from the resin blocks using an isomet 2000 linear precision saw. Sections were placed under a dissecting microscope and covered in methyl salicylate liniment APF to remove imperfections and cracks created by the saw. Each centrum was photographed under reflected light up to five times using a mounted camera. Images were edited using QuickTime (V.7.6.6) image capture software.

Age Estimation

Alternating opaque and translucent bands representing one band pair were visible in all samples with the exception of *U. asperrimus*. For this reason, no further analysis of this species was undertaken. The position of the birthmark in the section was evaluated from the angle change on the outer edge of the corpus calcareum

(Goldman 2005) (Figure 3.2A). Pre-birth banding was not present in any of the neonate samples; therefore the first band pair after the birth notch was regarded as age one. Age was determined by counting the band pairs on the outer edge of the corpus calcareum and 0.5 years was added if a translucent or opaque band was forming on the outer centrum edge (Smith *et al.* 2007; Pierce & Bennett 2009). Two training counts were conducted to achieve fluency in interpreting banding pairs and these scores were not included in the final results. Three counts were then made of each sample using three different readers. Final age estimates were achieved when the same age estimate was obtained from two or more readers. A qualitative readability score from one – three was given for each sample, where one meant all bands were clear and unambiguous; two, bands visible but difficult to interpret and three, bands were unreadable (modified from McAuley *et al*. 2006). Samples (n = 45) with readability scores of 3 were excluded from analyses. The index of average percentage error (IAPE) was calculated, after Beamish & Fournier (1981), to estimate the precision of age determination among readers. When averaged across multiple counts for multiple rays, the index provides an estimate of average percent error (Campana 2001). In addition, the coefficient of variation (CV) (Chang 1982) was also calculated.

Growth parameter estimation

I used a three-step approach to optimise the estimation of growth parameters. First, I pooled male and female samples and used Ford-Walford plots (Ford 1933; Walford 1946) to determine adequate starting values for estimating growth parameters. I used

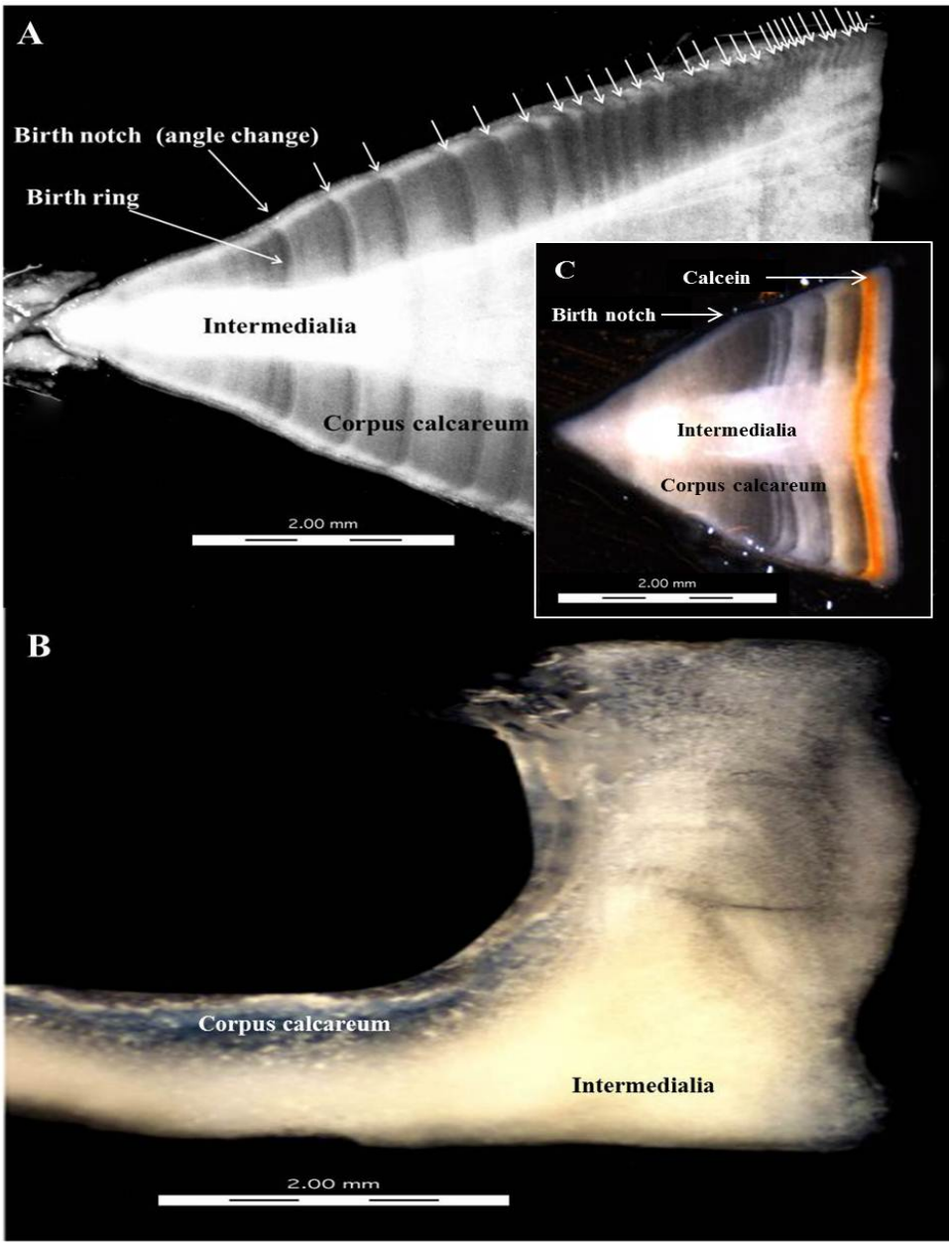


Figure 3.2: **(A)** Photographic images of sagittal centrum sections from *Pastinachus atrus* with 27 band pairs, **(B)** example of difficult to read section from *Urogymnus asperrimus* and **(C)** example of calcein marked centra from 91 days at liberty for *P. atrus*

maximum likelihood to compare a range of growth models to determine if a particular model best described the growth data (Cailliet *et al.* 2006).

Estimated ages and size measures (W_D), were fitted to four common models (Table 3.1): the three-parameter von Bertalanffy (VBGF) (Bertalanffy 1938), the modified two-parameter von Bertalanffy (2VBGF) (Fabens 1965), the logistic (after Smart *et al.* 2012) and the three-parameter Gompertz (GGF) (Ricker 1975). Akaike's information criterion (AIC) with a bias correction (AIC_c) due to small sample sizes (< 200) was used to determine best model fit (Zhu *et al.* 2009; Smart *et al.* 2012). Models were ranked according to AIC differences (Δ) where models with a Δ value of between zero – two were considered to have the highest support, while any higher Δ values were considered to have lower support (Burnham & Anderson 2002).

Table 3.1: Growth models and associated formulas used to fit size at estimated age data for four species of dasyatid ray

Model	**Growth Function**
3 parameter von Bertalanffy (VBGF)	$W_Dt = W_{D\infty}\,[1 - e^{-k\,(t-t0)}]$
2 parameter von Bertalanffy (2VBGF)	$W_Dt = W_{D\infty}\,(1 - be^{-kt})$, $b\ (W_{D\infty}\,W_{D0})/W_{D\infty}$
Logistic (LOG)	$W_Dt = \dfrac{W_{D\infty}\,W_{D0}\,e}{W_{D\infty} + W_{D0}\,(e^{(kt)-1})}$
Gompertz (GGF)	$W_Dt = W_{D\infty} = e\,((-W_{D0}\,e\,(-kt))$

Once the best model was determined, I adopted a Bayesian approach to approximate the posterior distribution of the growth parameters. Markov Chain Monte Carlo (MCMC) methods, using the Metropolis Hastings algorithm, were used to sample the posterior distributions (Hastings 1970; Gelman *et al.* 2003; Siegfried & Sansó 2006). I used a chain of two million iterations with a burn-in period of 100,000. Owing to the high auto-correlation in the MCMC chain, I used a thinning of 100. I used informative priors for $W_{D\infty}$ (asymptotic size expressed as disc-width) and k (growth coefficient – the rate at which asymptotic size was reached) based on published estimates for all available sub-tropical/tropical dasyatid species (n = 7) (Supp. Table 3.1 - Appendix 1). The prior for $W_{D\infty}$ was lognormal with mean 77 cm and standard deviation of 0.5 (in log space).

I used a beta distribution as a prior for k (Beta; 21.9; 162.3). The prior on the variance term was non-informative, defined by an inverse Gamma distribution (IGamma 0.01, 0.01). Preliminary sensitivity tests showed that using informative or non-informative priors for $W_{D\infty}$ and k did not affect the posterior estimates (Supp. Figure 3.1 - Appendix 1). Evidence of convergence of the MCMC chains was warranted by standard convergence diagnostics (visual inspection of the trace plots, the Geweke diagnostic test and from comparing summary statistics for the first 10% of the chain and the second half of the chain) (Supp. Figures 3.2 – 3.9 - Appendix 1). All analyses were conducted using the statistical package R (R Team Development Core 2011).

Results

Of the 170 rays sampled for age analysis, the vertebrae of 29% (n = 50) achieved readability scores of one, while 44% (n = 75) achieved scores of two, and 26% (n = 45) were assigned scores of three. The 13 *U. asperrimus* vertebral samples were excluded from analyses with only one sample attaining a readability score of < 3. In this species the cartilaginous matrix of the centra was very brittle for which it was very problematic to obtain accurate counts of band pairs (Figure 3.2B). The remaining samples that proved difficult to age were typically from very small individuals and full term pups, where calcification within the centra was insufficient to obtain counts. The index of average percentage error (IAPE) and coefficient of variation (CV) for remaining samples generally showed low inter-reader variability, particularly for the larger bodied species (*H. uarnak* and *P.atrus*) than the two smaller species (*N.kuhlii* and *T. lymma*) (Table 3.2).

Table 3.2: Index of average percentage error (IAPE) and coefficient of variance (CV) values for inter-reader precision of age determination (*i* = reader); * denotes larger bodied species, ** smaller bodied species

Species	IAPE *i*=1	IAPE *i*=2	IAPE *i*=3	CV *i*=1	CV *i*=2	CV *i*=3
Himantura uarnak*	1.69	1.66	2.92	2.64	2.29	4.03
Neotrygon kuhlii	2.19	2.84	5.25	3.12	4.04	7.47
Pastinachus atrus*	1.43	2.45	4.66	2.07	3.55	6.75
Taeniura lymma	2.09	2.15	3.61	2.80	2.88	4.84

Recaptures and seasonal edge deposition

Of the 52 rays caught and marked with calcein, two were recaptured after 83 and 91 days at liberty since November 2009. These rays had both grown 5 cm (W_D) during this period and both had laid down translucent bands of 0.2 cm in width with a very pronounced calcein mark (Figure 3.2C). Unfortunately, the time at liberty was insufficient for the validation of band pair periodicity. Variation in sampling effort resulted in 48 rays being caught in summer and 77 in winter. Of the 48 individuals caught in February, 28 (58%) had opaque bands forming at the edge of the centra, while during the winter months, 51 rays (66%) had translucent bands forming on the centrum edge.

Comparison of growth models

Estimated ages ranged from one to 25 years for *H. uarnak* (25—145.4 cm W_D), from 1.5 to 13 years in *N. kuhlii* (17—47 cm W_D), from one to 27 years in *P. atrus* (36.5—177 cm W_D), and from one to 11 years in *T. lymma* (14—34.5 cm W_D) (Figure 3.3). The 2VBGF provided the best fit and realistic parameter values for all species (Table 3.3 and Figure 3.3). The remaining three models failed to provide reliable estimates.

Table 3.3: Comparison of growth model fit and parameter estimates

Species	Model	ΔAIC	$W_{D\infty}$ (cm)	k (yr $^{-1}$)	T_0 (cm)
Himantura uarnak	VBGF	4.43	137.11	0.08	-1.29
n = 19	GGF	10.42	131.45	35.66	-1.05
	LOG	7.18	91.26	16.28	24.58
	2VBGF	0	144.67	0.05	-
Neotrygon kuhlii	VBGF	4.44	39.22	1.12	0.68
n = 34	GGF	4.45	39.17	1.29	0.98
	LOG	4.46	39.13	1.48	5.46
	2VBGF	0	41.63	0.39	-
Pastinachus atrus	VBGF	4.08	166.81	0.12	-1.25
n = 32	GGF	3.91	151.47	0.24	2.12
	LOG	4.91	73.77	13.97	18.2
	2VBGF	0	155.03	0.16	-
Taeniura lymma	VBGF	4.36	29.47	0.56	-0.22
n = 40	GGF	4.39	29.15	0.73	0.5
	LOG	4.42	28.93	0.92	8.29
	2VBGF	0	32.14	0.25	-

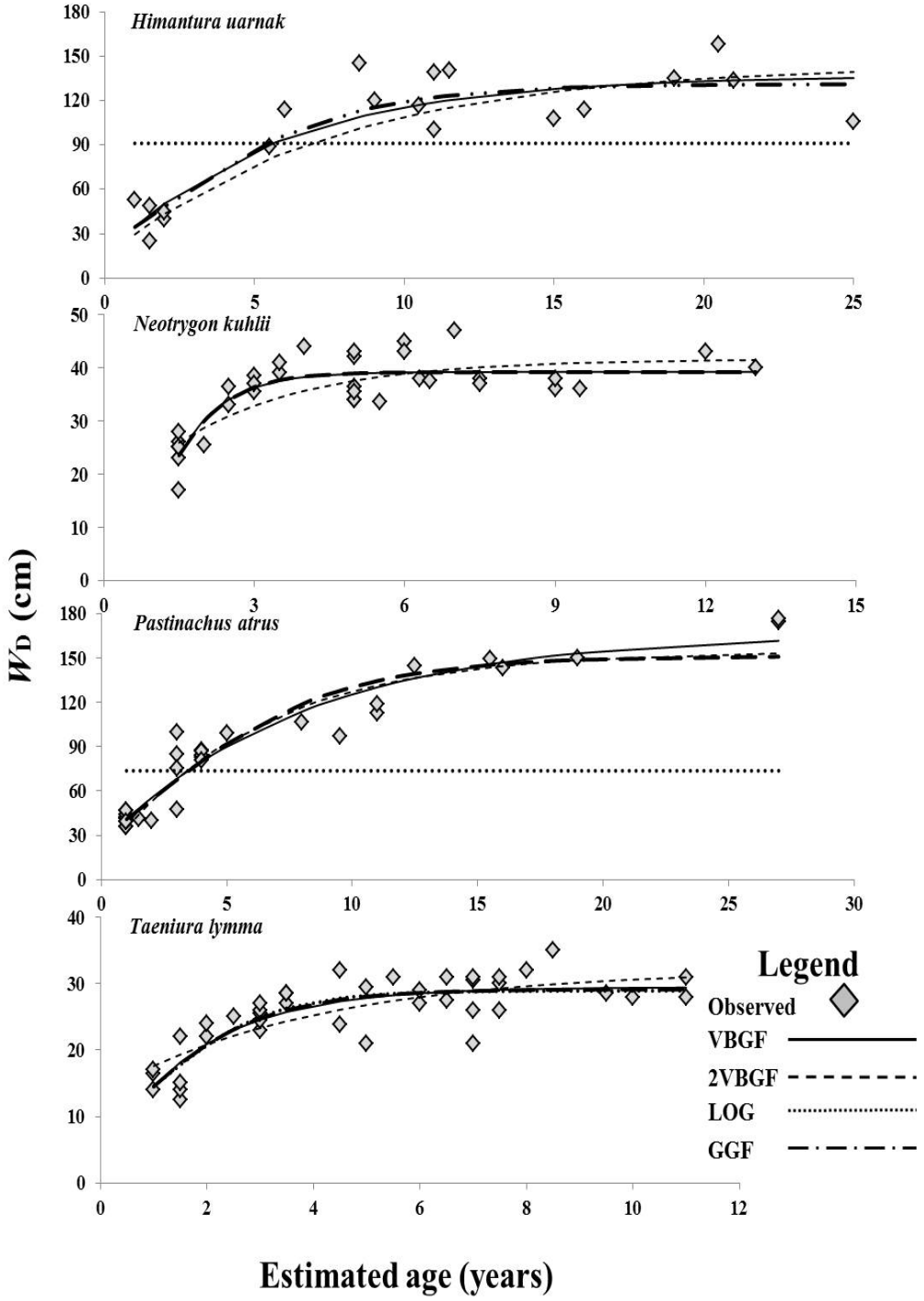


Figure 3.3: Observed and predicted age at size (disc width, cm) for the different growth models VBGF, 2VBGF, LOG and GGF

Estimation of growth parameters

Diagnostic tests indicated MCMC chain convergence for all growth parameters for three species (*N. kuhlii, P. atrus and T. lymma*). Convergence for $W_{D\infty}$ for *H. uarnak* was less obvious, reflecting the less than ideal characteristics of the data (i.e. few large/old individuals). Growth data were informative for all species, updating the priors for k and $W_{D\infty}$ in all cases (Figure 3.4).

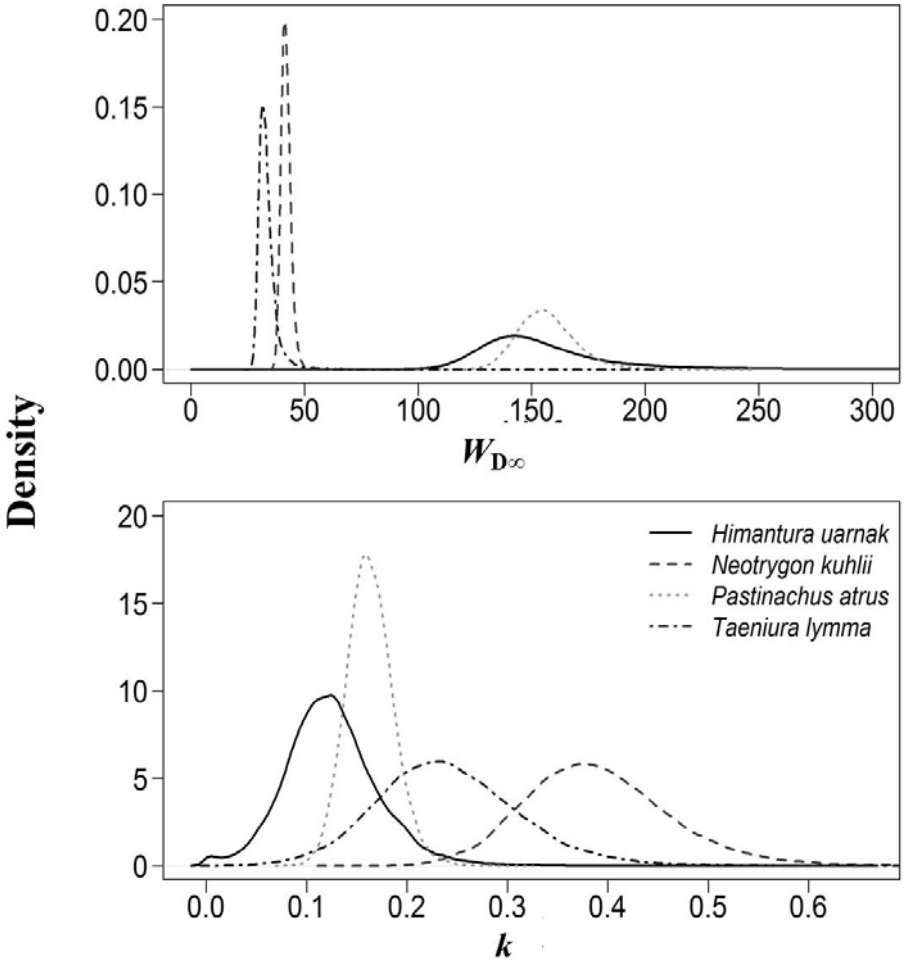


Figure 3.4: Posterior distributions for $W_{D\infty}$ and k, for the four species where band pairs could be counted

The Bayesian approach provided more precise estimates of *k* (median with 95% credibility intervals for *P. atrus* (*k* = 0.16, 0.12 – 0.21) than for the remaining species (*H. uarnak: k* = 0.12, 0.04 – 0.22; *N. kuhlii*: *k* = 0.39, 0.25 – 0.53; and *T. lymma*: *k* = 0.24, 0.1 – 0.38). For $W_{D\infty}$, more precise estimates were obtained for *N. kuhlii*, ($W_{D\infty}$ = 41.74 37.80 – 46.11) and *T. lymma*, ($W_{D\infty}$ = 32.64 27.62 – 40.73) than for *P. atrus* ($W_{D\infty}$ = 155.69 133.03 – 181.35) and *H. uarnak* ($W_{D\infty}$ = 148.95 107.24 – 230.53), with the latter showing a much broader posterior distribution (Table 3.4 and Figure 3.4).

Table 3.4: Summary of growth parameter estimates (median with 95% credibility intervals) from the Bayesian modelling approach

Species	$W_{D\infty}$ (cm) ± 95% C.I.	*k* (yr $^{-1}$) ± 95% C.I.
Himantura uarnak	148.95 (107.24 – 230.53)	0.12 (0.04 – 0.22)
Neotrygon kuhlii	41.74 (37.80 – 46.11)	0.38 (0.25 – 0.53)
Pastinachus atrus	155.69 (133.03 – 181.35)	0.16 (0.12 – 0.21)
Taeniura lymma	32.64 (27.62 – 40.73)	0.24 (0.1 – 0.38)

Discussion

My results show that realistic growth parameters can be estimated in data-limited situations by the use of a combination of statistical methods. This is particularly relevant for rare or protected species, for ecosystems characterised by very high species diversity, or for protected areas where large sample sizes cannot be obtained

using conventional techniques. A key element of this result was that despite being few in numbers, my samples included a wide range of size classes, enabling the estimation of growth parameters without the need to resort to other methodologies (e.g. back calculation).

Validation of age estimates

There have been very few studies of the age and growth of tropical dasyatids, with only one validating the annual patterns of deposition of band pairs (Pierce & Bennett 2009). Although I attempted age validation through the recapture of chemically-marked individuals (Campana 2001) I failed to obtain any rays after a sufficient period at liberty. For this reason I was forced to assume that the band pairs I counted were deposited on an annual basis. This appears reasonable, given that annual patterns of deposition have been reported for the majority of elasmobranchs examined to date.

Growth models and parameter estimates

The two-parameter vonBertalanffy function (2VBGF) provided the best fit to age and size data sets of all species. Parameter estimates from this model were comparable to published estimates for other tropical dasyatid rays (e.g. Cowley 1997, Ismen 2003, Smith *et al.* 2007, Jacobsen & Bennett 2010, Jacobsen & Bennett 2011, Dale & Holland 2012). Given that L_0 is generally well documented for sharks and rays, the use of the 2VBGF – where only the k and $W_{D\infty}$ parameters were estimated – is preferable over the traditional VBGF model when sample sizes are

small (Cailliet & Goldman 2004; Thorson & Simpfendorfer 2009). In addition to k and $W_{D\infty}$, the use of VBGF requires estimation of T_0, which is often misinterpreted as the length of gestation, but in reality has no biological interpretation (Cailliet & Goldman 2004). Due to the requirement of estimating an additional parameter and the highly correlated nature of growth parameters, it is not surprising that the 2VBGF outperformed the other models. Hence, my study clearly supports the use of the 2VBGF model in data-limited cases.

Studies conducted on related species suggest that no particular growth model is better at describing the growth of dasyatids. For example, for brown stingrays, *Dasyatis lata,* the LOG growth model provided best fit (Dale & Holland 2012), whereas the growth of the black whipray, *Himantura astra* (Jacobsen & Bennett 2011) and the diamond stingray, *Dasyatis dipterura* (Smith *et al*. 2007) was better described by GGF and the VBGF respectively. Growth rates are defined by the growth coefficient (k) which describes the rate at which asymptotic growth occurs (Hoenig & Gruber 1990). Slow-growing elasmobranchs are reported as having k values < 0.1 (Branstetter 1990) and it is therefore assumed that these species are more vulnerable to extrinsic pressures such as overfishing (Musick 1999) than those species where $k > 0.1$. Hence, my findings suggest that of the two largest species I studied, *H. uarnak* is more vulnerable as a slower-growing species ($k = 0.12$), than *P. atrus* ($k = 0.16$), though their posterior distributions overlap considerably. As might be expected, the two smaller-bodied species (*N. kuhlii* and *T. lymma*) have faster growth rates ($k = 0.38$ and 0.24 respectively), and are thus less vulnerable.

Published studies for other sub-tropical/tropical dasyatid species show similar results, with those species attaining larger maximum sizes ($W_D max$ > 100 cm) having slower growth rates than smaller-bodied species (e.g. Jacobsen & Bennett 2011, Yigin & Ismen 2012).

Conclusions

My study introduces a multi-staged approach for estimating growth parameters in data poor situations. My results are comparable to those of earlier studies of related species, despite data limitations. Furthermore, my results suggest that the 2VBGF model is most appropriate for use in cases where data are limited and size at birth information is available, since traditional three-parameter growth models tend to perform relatively poorly. Finally, my approach makes use of all available information in the form of Bayesian priors, providing more robust growth estimates than standard regression approaches when data are limited (Siegfried & Sansó 2006).

The development of alternative, viable methods for estimating growth parameters of elasmobranchs when sample sizes are small and not ideally represented is critical for determining the vulnerability of rare and protected species, or any species where the collection of large sample sizes presents logistic or ethical problems. By combining different analytical methods and maximising the use of available information, my approach can increase the precision in estimating growth parameters of such species.

Chapter 4:

Dietary partitioning by five sympatric stingrays (Dasyatidae) at Ningaloo Reef, Western Australia

Introduction

Mesopredators provide the connection between apex predators and lower trophic levels of food chains in biological communities (Vaudo & Heithaus 2011). In coastal marine environments, batoid fishes are important mesopredators that can make up a significant part of the biomass of the fish community (VanBlaricom 1982; Thrush *et al.* 1994; Peterson *et al.* 2001; Vaudo & Heithaus 2011) and their feeding can regulate the numbers and dynamics of invertebrate prey populations (Wetherbee & Cortés 2004; Navia *et al.* 2007).

Coastal environments of the tropics are characterised by very high diversity and abundance of rays (Last & Stevens 2009). Given their role as structuring agents, this observation prompts the question as to how so many of these mesopredators can co-exist in these systems. Theory predicts that where many similar species occupy the same ecosystem, resources should be partitioned along some ecological axis (typically space, time or food) within the environment so that competition among them is minimised, avoiding competitive exclusion and thus allowing co-existence (Schoener 1974). There is some evidence that this phenomenon occurs in rays, with studies showing that ecosystems can be partitioned among species by occupation of different depth ranges (Babel 1967; Marshall *et al.* 2008), habitats within an environment (White *et al.* 2004; Marshall *et al.* 2008) or by specialisation in diet (Platell *et al.* 1998*a*).

Chapter 4: Dietary Partitioning

An understanding of the trophic roles and resource partitioning of rays is critical to conservation strategies, ecosystem-based management and for predicting impacts associated with species removal (Yick *et al.* 2011). Such information is urgently required for tropical regions where rays are targeted or important by-catch in many coastal fisheries. In addition, coastal ecosystems border large and growing human populations, so that anthropogenic disturbance resulting in habitat loss or degradation is commonplace (Halpern *et al.* 2007). Furthermore, the conservative life history traits of rays (slow growth, low reproductive capacity, late age at maturity) means that populations have low resilience and may require many years to recover from over-exploitation (Stevens *et al.* 2000).

Here, the diets of five species of sympatric batoids were assessed within the lagoon of a coral reef ecosystem at Ningaloo Reef, Western Australia. Although rays are common in these habitats (Stevens *et al.* 2009), my study is one of the first to examine the feeding biology and dietary preferences of these animals within a coral reef environment. Recent work in these reef systems shows that some parts of the lagoon are subject to intense feeding by rays that is capable of overturning and re-working large areas of sediment (O'Shea *et al.* 2012). Stomach content analysis was used to identify the targets of this foraging behaviour and examine the degree to which rays that inhabit the lagoon divide food resources. It is predicted that these sympatric rays should partition diets according to the degree of overlap in the habitats that they occupy, so that species that co-occur on most spatial and temporal scales should show the lowest dietary overlap.

Material and Methods

Study location and species

This study was conducted between February 2010 and February 2011 at 18 sites across the northern, middle and southern sections of Ningaloo Reef (Figure 4.1). This reef is the largest fringing coral reef in Australia occupying a stretch of approximately 250 km of the northwest coast of the continent. The lagoon is generally shallow (≤ 10 m water depth) and the reef crest varies in distance from the shore from 100 m – 4 km (Stevens *et al.* 2009). The convoluted coastline provides a range of diverse habitats including mangroves, rocky shores and coral reefs. Sites were chosen based on both ease of access and prior surveys that identified areas of high ray abundance.

170 rays of five species were caught, including the blue-spotted mask *Neotrygon kuhlii* (Müller & Henle 1841) (n = 36), cowtail *Pastinachus atrus* (Macleay 1883) (n = 43), blue-spotted fantail *Taeniura lymma* (Forsskål 1775), (n = 54) porcupine *Urogymnus asperrimus*, (Bloch & Schneider 1801), (n = 13) rays and the reticulate whipray *Himantura uarnak* (Forsskål 1775), (n = 24). Rays were caught in water depths ranging from 0.5—10 m in sandy lagoon habitats. Small rays (< 100 cm W_D) and those close to shore were caught using hand and seine nets. Larger rays (> 100 cm W_D) were caught using spear guns and then brought to shore for dissection. Body mass was measured for each ray using a spring balance (± 100 g) and disc width using a tape measure (± 5 mm). Sexual maturity of each ray was assessed by calcification of claspers in males and macroscopic assessment of females.

including egg presence and uterine scarring, as well as published estimates of size at maturity from Last & Stevens (2009). To assess prey selectivity, 225 sediment cores were taken (400 cm^3 each) using 400 ml plastic sample containers (16 cm × 5 cm × 5 cm) that equated to a volume of ~ 90,000 cm^3 (90 litres). These samples were collected between April 2010 and April 2011from 10 sites at Mangrove Bay within the northern most sampling site (Figure 4.1) that had been identified as areas used by rays for foraging (O'Shea *et al.* 2012). Samples were stained with Rose Bengal and stored for 24 hours prior to sorting to ensure sufficient staining of infauna within the sediment (after methods outlined in Mason & Yevich 1967). To separate infauna, each sample was washed through a 45-µm sieve using distilled water. All taxa were identified to the highest possible taxonomic resolution using a dissecting microscope. There was no distinguishing between epibenthic fauna and infauna and for this reason, all prey taxa discussed hereafter are referred to as infauna.

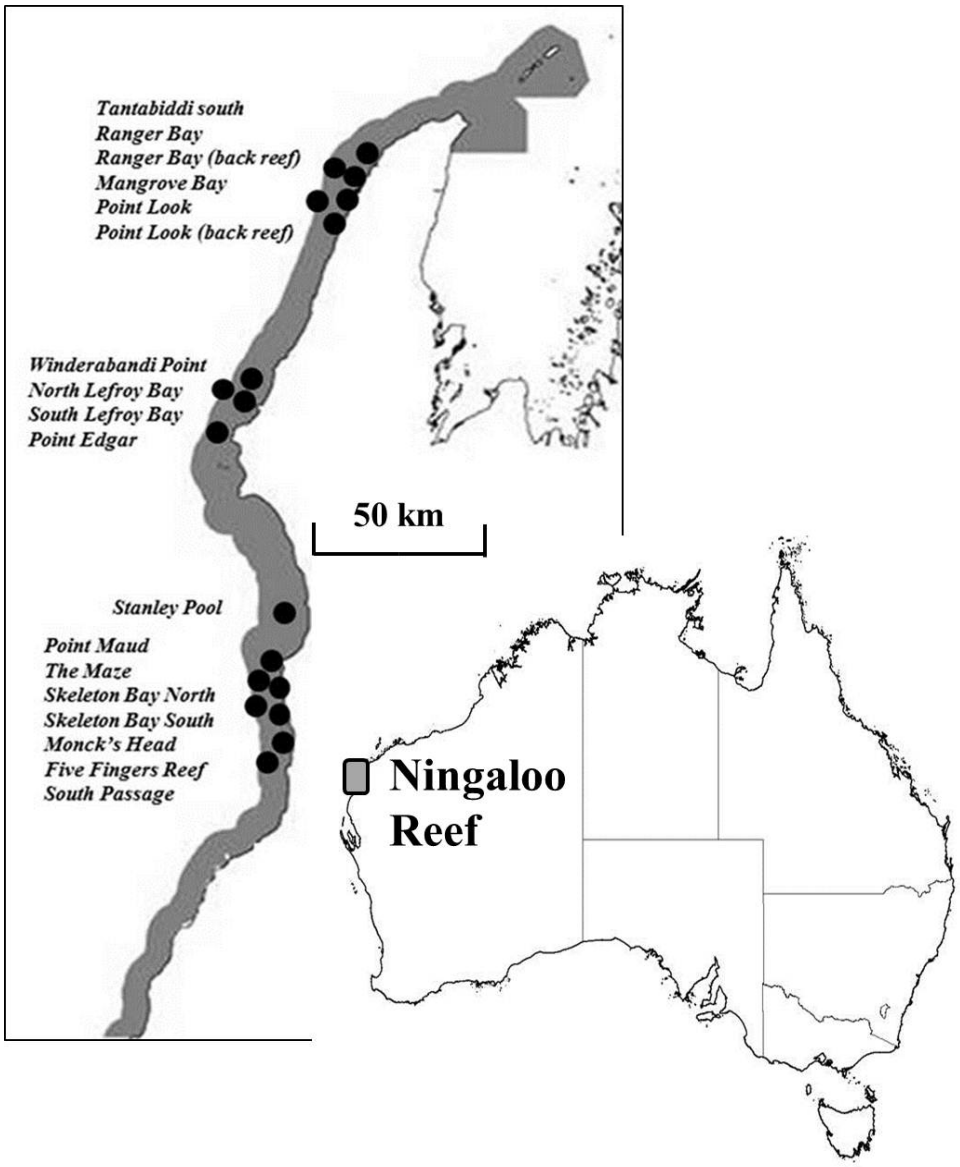


Figure 4.1: Map showing Ningaloo Reef and the 18 sites where rays were collected. The shaded area shows the extent of the marine park

Diet analysis

Stomachs were dissected from each ray and its contents along with any items present in the buccal cavity and oesophagus were extracted and stored in sealable plastic bags and frozen. Prey items were identified using a dissecting microscope when

required Cumulative prey curves were generated to assess whether the number of stomachs analysed was sufficient to characterise the diet. This was done by plotting the cumulative number of new prey items found for each new stomach analysed for each species and sex and visually determining whether the curves reached an asymptote (Ferry & Cailliet 1996).

Data Analysis

Data were pooled into 10 taxonomic categories due to the large number of zeros in the data set. In order to avoid biases involved in the use of a single index, the contribution of each prey item to the dietary composition of each ray species was determined using three indices; the relative abundance of prey taxa (%n), calculated as the number of prey items of a given prey category / total number of prey items for all prey categories × 100 (Hyslop 1980), the percentage frequency occurrence (%FO), calculated as the total number of stomachs containing an item belonging to an individual prey category / total number of stomachs sampled that were not empty × 100 (Vaudo & Heithaus 2011) and the prey-specific abundance (%P*i*), calculated as the number of prey items of a given prey category / total number of prey items in only those rays with the given prey category in their stomachs (Amundsen *et al.* 1996). Abundance estimates (as opposed to percentage weights or volumes) were considered sufficient for this task because my aims were to compare diets among ray species rather than assess calorific intake or nutritional value of prey.

Feeding strategies of each of the five rays were described by plotting %FO versus %P*i* (Costello 1990; Amundsen *et al*. 1996). These plots demonstrate the

importance of a prey category as a component of diet, the feeding strategy (generalist or specialist) as well as assessing inter- and intra-individual niche width. The vertical axis represents the feeding strategy (specialisation or generalisation) of the predator. Points positioned in the upper part of the graph indicate specialist prey items, whereas points in the lower part indicate items that were only eaten occasionally. Dietary items falling in the upper left of the diagram indicate specialisation by individual predators, and those in the upper right indicate specialisation by the entire population. If most points are located on the upper right of the diagram, this reflects a predator population with a specialised feeding strategy (i.e. a narrow niche width). If no points are located in the upper right of the diagram and all points fall along or below the diagonal from the upper left to the lower right, the predator population is thought to have a generalist diet and thus a broad niche width. The distribution pattern of dietary items along the diagonal from top left to bottom right of the plot is indicative of the contributions of between- and within-phenotype components to the niche width, where points falling on the top left indicate the former and bottom right the latter phenotypes (see Amundsen *et al.* 1996).

The Manly-Chesson index (α) was calculated (Manly 1974; Chesson 1978) to assess prey selectivity:

$$\alpha = (r_i/p_i) / \sum_{i=1}^{m} ri\ /pi,$$

where r_i = proportion of prey category i in the stomach content, p_i = proportion of prey category i in the environment and m = the number of prey categories present in the environment. Values of α range between 0 (complete avoidance) and 1 (complete

preference). Dietary overlap was also assessed using Morisita's index ($C = (2\sum X_i Y_i) / \sum X^2 + \sum Y^2$) (Morisita 1959) where X_i and Y_i are proportions of the ith prey category in the stomach content of species X and Y respectively. Values of C range from 0 (no overlap) to 1 (complete overlap). Overlap of ≥ 0.6 is considered a strong competitive interaction between species (Zaret & Rand 1971; Järv *et al.* 2011).

Non-metric multidimensional scaling analysis (MDS) was used to visualise variation in diet composition and analysis of similarity (ANOSIM) to test for differences in diet among all ray species and between sexes and ontogenetic stages of individual species. All data were square root transformed to reduce the influence of prey with high abundances. The Bray-Curtis coefficient was used to calculate the similarity matrix and the resulting scaling ordinations were considered useful interpretations if stress values were < 0.2 (Clarke & Gorley 2006). Percentage similarity in diets among groups was also assessed using similarity percentages (SIMPER). All analyses were conducted using PRIMER V6 (Clarke & Gorley 2006). Ontogenetic differences (adult v juvenile rays) in diet were only assessed for *H. uarnak* and *P. atrus* due to low sample sizes of juveniles. If a significant difference ($P < 0.05$) in the global ANOSIM was found, pair-wise tests were then examined. The significance levels were not used solely for interpretation of pairwise tests as in this context, P values are highly dependent on the number of replicates in the comparison. For this reason, the R-values are considered more reliable for interpretation (Clarke & Gorley 2006). The R statistic varies from 0 (no difference) to 1 (complete dissimilarity).

Results

Abundance of prey taxa

Of the 170 ray stomachs sampled, 28% were empty (22% of *N. kuhlii*, 8% of *H. uarnak*, 44% of *P. atrus*, 35% of *T. lymma* and 15% of *U. asperrimus*). Cumulative prey curves indicated that my sample sizes were sufficient to give an accurate representation of diets of *H. uarnak, N. kuhlii* and possibly *P. atrus and T. lymma* (Figure 4.2). Prey curves for *U. asperrimus* suggested more samples were needed, although numbers appeared very close to sufficient to characterise diet. A broad range of sizes (disc widths) were sampled for each species, however for ontogenetic comparisons, prey were only recovered in sufficient numbers for analysis from juveniles and adults of *H. uarnak,* and *P. atrus*. Juveniles of both *T.lymma* and *N. kuhlii* had either too few samples or stomachs were empty of prey. All *U. asperrimus* sampled were adults. In total, 2,804 individual prey items from 24 taxa were identified and diets were dominated by annelids, penaeid prawns, other prawns, brachyurans and bivalves; together these taxa accounted for 96% of total prey recovered from stomach samples (Table 4.1 and Supp. Table 4.1 - Appendix 2). Annelids dominated the diet of all ray species except *H. uarnak*, whose diet was dominated by penaeid prawns (Table 4.1 and Supp. Table 4.1 - Appendix 2). Sediment samples contained 3,215 individual taxa, and were dominated by annelids (55%), gastropods (31%), bivalves (8%), echinoderms (4%) and prawns (1%) (Supp.Table 4.2 - Appendix 2).

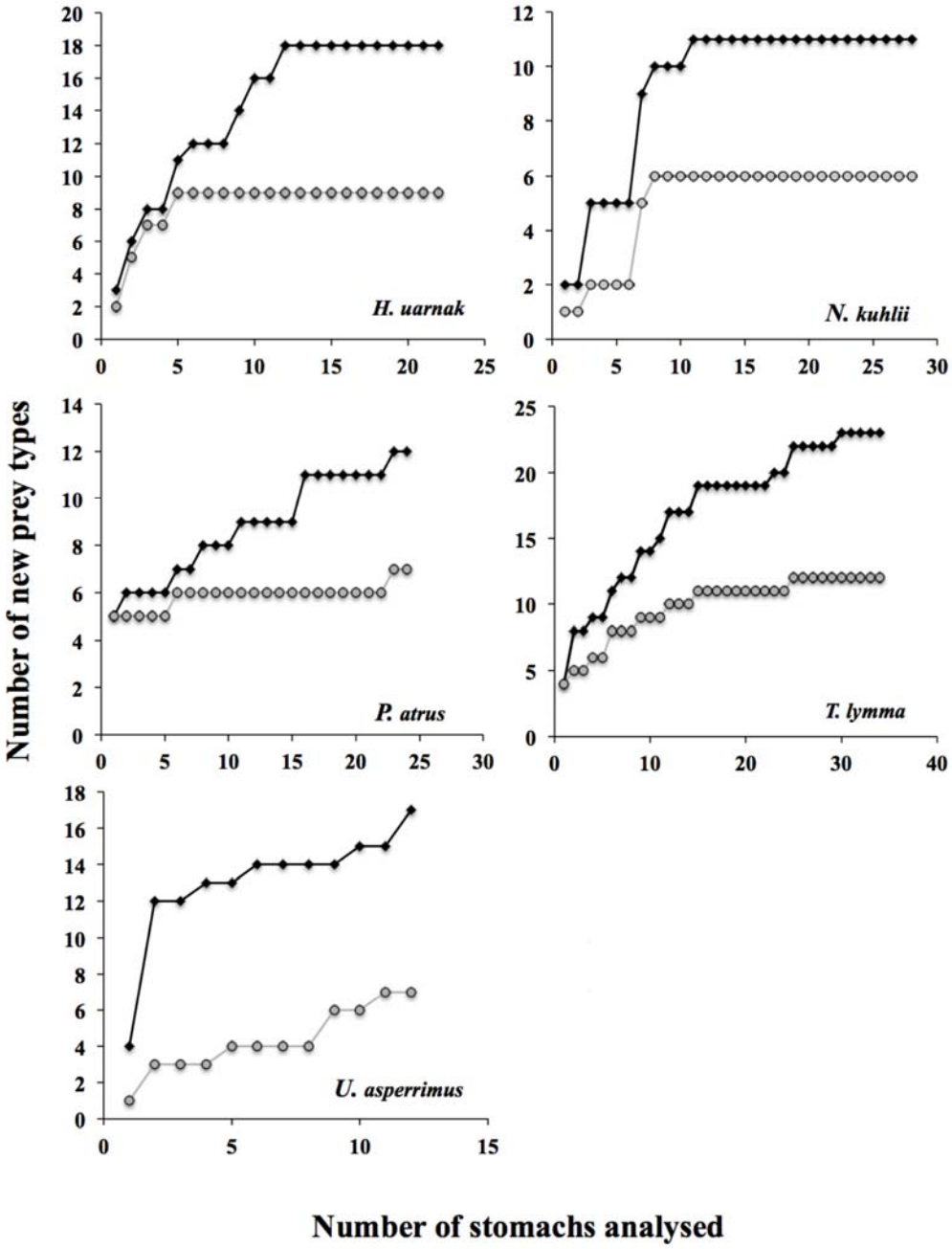


Figure 4.2: The cumulative number of new prey taxa for each consecutive stomach sampled for all prey taxa found (black line) and for the 10 summarised prey categories (grey line)

The ANOSIM found significant differences in the diet composition among rays (Global R statistic = 0.12; P = 0.002) (Figure 4.3). Pairwise tests from the ANOSIM indicated that the diet of *H.uarnak* was dissimilar to all other species, except *U. asperrimus* and was most dissimilar to *N.kuhlii* (R = 0.42, P = 0.001) (Table 4.2). The next greatest dissimilarity occurred between the diets of *N. kuhlii* and *U. asperrimus*, (R = 0.31, P = 0.005) (Table 4.2). SIMPER analysis revealed that the main prey categories contributing to dissimilarity of diets of the rays was annelids and penaeid prawns. The average Bray-Curtis dissimilarity between *H. uarnak* and *N. kuhlii* was 78.94 and this was made up of annelids (26.53%), penaeid prawns (26.06%), brachyurans (15.99%) and other prawns (12.16%). Annelids were much lower in abundance in the diet of *H. uarnak* compared to *N. Kuhlii* and penaeid prawns, brachyurans and other prawns were much higher in abundance in *H. uarnak* (Figure 4.4). The average Bray-Curtis dissimilarity between *N. kuhlii* and *U. asperrimus* was 62.67 and this was made up of annelids (45.68%), penaeid prawns (19.44%) and other prawns (11.46%). Even though annelids were the main contributing dietary item to both *N. kuhlii* and *U. asperrimus*, annelids were much higher in average abundance in *U. asperrimus* compared to *N kuhlii.* Additionally *U. asperrimus* also had higher contributions of penaeid prawns and other prawns compared to *N. kuhlii* (Figure 4.4).

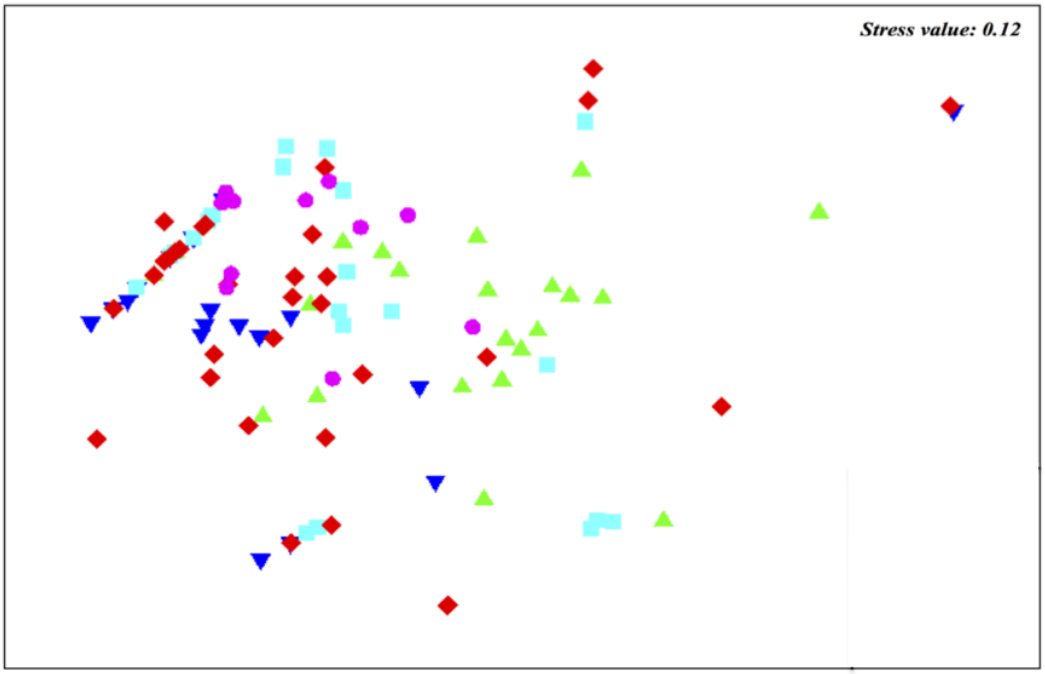


Figure 4.3: Non-metric multidimensional scaling ordination of rays based on stomach content

Feeding strategy plots further verified that penaeid prawns were an important prey category for *H. uarnak*. In addition, there was a moderate level of specialisation by *H. uarnak* on penaeid prawns with relatively narrow niche width (Figure 4.5). The Manly-Chesson analysis suggested that *H. uarnak* fed selectively on brachyurans ($\alpha = 0.58$), which were rare in the cores, while annelids, the most common prey item in the cores were consumed with low preference ($\alpha < 0.01$). This species fed with a moderate preference for penaeid prawns ($\alpha = 0.14$) and other prawns ($\alpha = 0.21$) (Table IV), both of which were relatively rare in the cores (~ 1%). Morisita's index of overlap between this species and the other rays suggested some competitive interaction with moderate dietary overlap: *N. kuhlii* ($C = 0.44$), *P. atrus* ($C = 0.57$), *T. lymma* ($C = 0.49$) and *U. asperrimus* ($C = 0.62$).

Table 4.1: Summary of prey taxa consumed by *Himantura uarnak*, *Neotrygon kuhlii, Pastinachus atrus*, *Taeniura lymma* and *Urogymnus asperrimus* from Ningaloo Reef, quantified by dietary measures: overall sampled size (n), percentage of numerical importance within each species (%n), and percentage frequency of occurrence (%FO)

	H. uarnak		*N. kuhlii*		*P. atrus*		*T. lymma*		*U. asperrimus*	
Prey Category	**%FO**	**%n**	**%FO**	**%n**	**%FO**	**%n**	**%FO**	**%n**	**%FO**	**%n**
Annelids	50.00	22.44	85.71	81.82	70.83	69.65	73.53	69.01	100.00	70.52
Bivalves	31.82	6.43	25.00	2.39	12.50	5.08	23.53	7.03	18.18	1.78
Brachyurans	63.64	16.14	50.00	4.31	29.17	10.56	8.82	0.57	36.36	2.13
Cephalopods	22.73	2.49	17.86	0.00	4.17	0.13	2.94	0.19	9.09	0.36
Copepods	0.00	0.00	0.00	0.00	0.00	0.00	5.88	1.14	0.00	0.00
Fishes	22.73	1.57	17.86	0.48	0.00	0.00	5.88	0.19	0.00	0.00
Gastropods	4.55	0.52	3.57	0.00	8.33	0.53	5.88	0.76	0.00	0.00
Other prawns	63.64	9.45	50.00	9.57	33.33	5.35	41.18	12.17	36.36	5.86
Penaeid prawns	63.64	40.55	7.14	1.44	29.17	7.62	20.59	3.80	0.00	16.70
Priapulids	4.55	0.39	0.00	0.00	4.17	1.07	11.76	4.37	36.36	2.66

The abundance of annelids in the diets of the four remaining species resulted in high dietary overlap between all other species pairs ($C = 0.98$). Feeding strategy plots suggested that annelids were not only a dominant part of the diet of all four species, but also there was a high degree of specialisation on this prey item (Figure 4.5). As with *H. uarnak*, the feeding strategy plots suggested a narrow niche width for these species. The Manly-Chesson index of selectivity suggests that annelids were being consumed with low selectivity when compared to the high environmental availability (Table 4.3).

Table 4.2: Summary of results from the ANOSIM pairwise tests for differences between species

	H. uarnak	***N. kuhlii***	***P. atrus***	***T. lymma***	***U. asperrimus***
H. uarnak	-	P < 0.01	P < 0.01	P < 0.01	P = 0.07
N. kuhlii	R = 0.42	-	P < 0.01	P = 0.08	P < 0.01
P. atrus	R = 0.14	R = 0.14	-	P = 0.29	P = 0.99
T. lymma	R = 0.20	R = 0.04	R < 0.01	-	P = 0.81
U. asperrimus	R = 0.10	R = 0.31	R = 0.12	R = 0.09	-

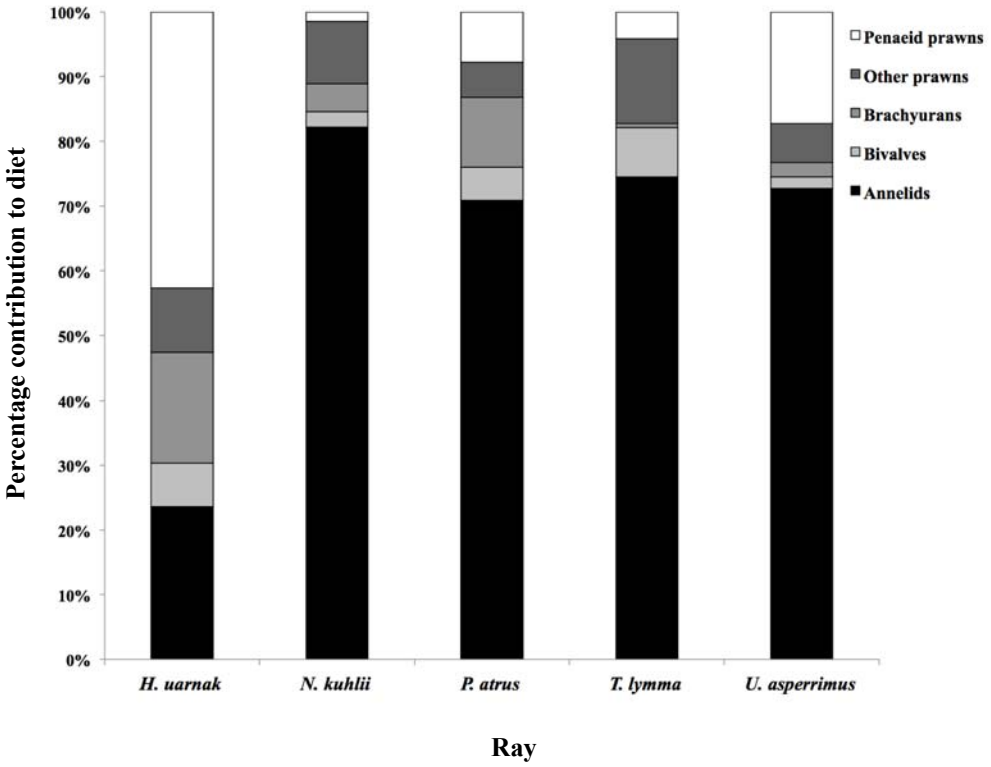


Figure 4.4: Percentage contributions of the most dominant prey categories to the diets of each ray species.

Dietary shifts relating to sex and ontogeny

Only *N. kuhlii* displayed differences between the diets of males and females (ANOSIM, Global R statistic = 0.16; P = 0.036). The average Bray-Curtis dissimilarity in diets between males and females was 48.44 and this difference was predominantly due to annelids (54.83%) and other prawns (23.47%), which occurred in slightly higher abundances in the diets of females. There were significant differences in the diets of juvenile and adult *H. uarnak* (Global R statistic = 0.30; P = 0.016) and *P. atrus* (Global R statistic = 0.15; P = 0.037). The average Bray-Curtis

dissimilarity between diets of juvenile and adult *H. uarnak* was 76.11 and this difference was predominantly made up of penaeid prawns (26.76%), annelids (20.33%) and brachyurans (15.03%) that occurred in slightly higher abundances in the diet of adults than in juveniles.

The average Bray-Curtis dissimilarity in diet between juveniles and adults for *P. atrus* was 71.94 and this difference was predominantly due to annelids (50.09%), brachyurans (15.96%), penaeid prawns (12.27%) and other prawns (11.86%), that were in higher abundances in the diets of adults.

Table 4.3: Values of α calculated using the Manly-Chesson index of prey selectivity. Values range between 0 (complete avoidance) and 1 (complete preference)

	Annelids	**Bivalves**	**Brachyurans**	**Fish**	**Gastropods**	**Other prawns**	**Penaeid prawns**
H. uarnak	< 0.1	< 0.1	0.58	< 0.1	< 0.1	0.21	0.14
N. kuhlii	< 0.1	< 0.1	0.4	< 0.1	na	0.53	< 0.1
P. atrus	< 0.1	< 0.1	0.72	na	< 0.1	0.23	< 0.1
T. lymma	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.85	< 0.1
U. asperrimus	< 0.1	< 0.1	0.29	na	na	0.47	0.23

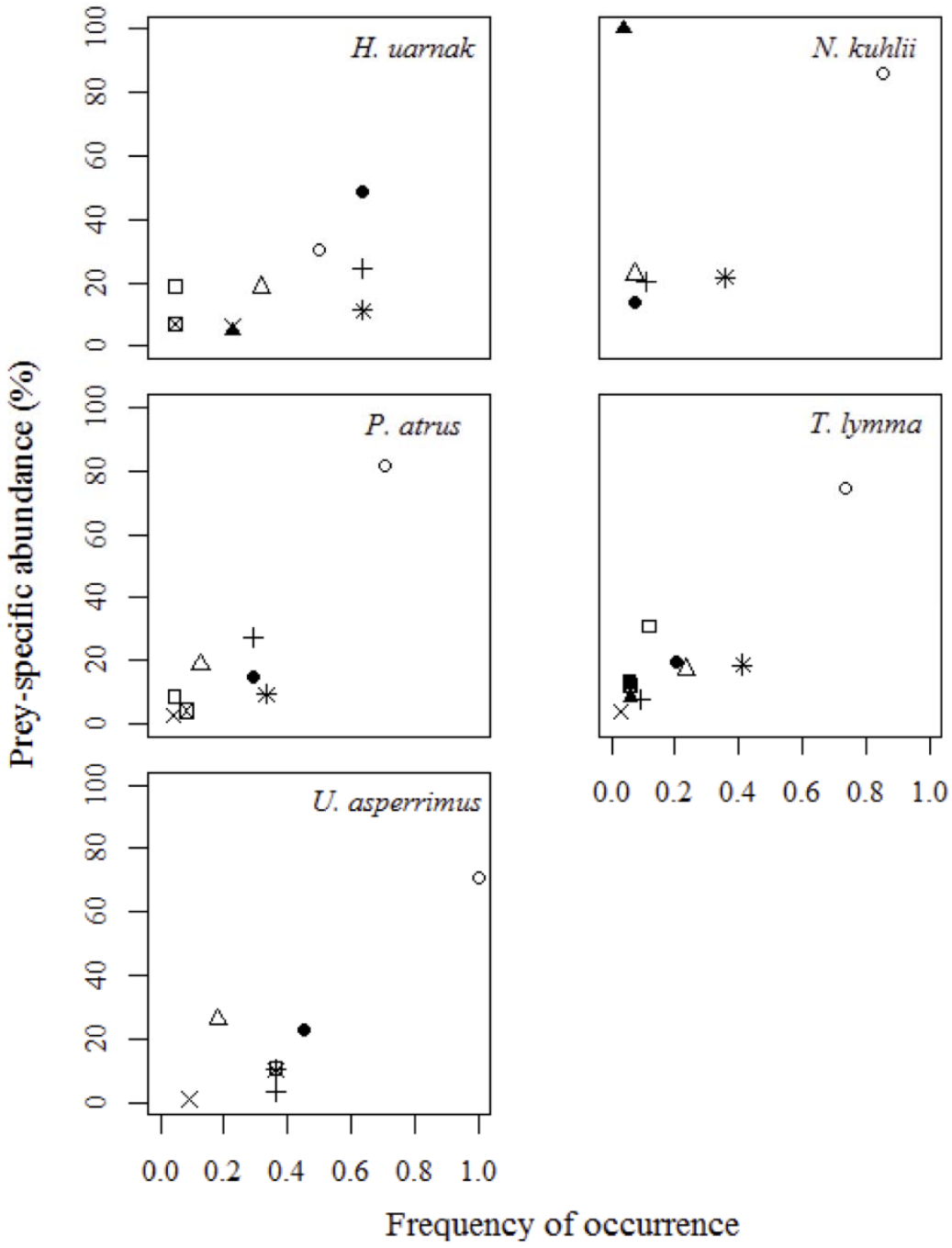


Figure 4.5: Feeding strategy plots for each of the five species of ray sampled

Discussion

There was limited evidence for dietary partitioning among and within species of ray in the lagoon of Ningaloo Reef. With exception of *H. uarnak*, which had a diet largely composed of crustaceans; the diets of *P. atrus, N. kuhlii T. lymma* and *U. asperrimus* were all dominated by annelids. For all rays, the combined abundance of only four taxonomic groups (annelids, prawns, brachyurans and bivalves) accounted for over 96% of all prey items, implying that they all probably occupy similar trophic positions in the lagoon food web.

As my analysis was based on abundance, it might be possible that smaller, numerically dominant prey might overshadow the contribution of larger but less abundant prey items. However, the principal categories of prey were common to all the diets of all species in quantities that suggested calorific significance, rather than incidental consumption. Furthermore, my results confirm those of Vaudo & Heithaus (2011) who used indices that combined both biomass and abundance to characterise the diet of some of the same species in Shark Bay, to the south of Ningaloo Reef. As was the case in my study, they found that brachyurans and penaeid prawns dominated the diet of *H. uarnak*, while the diet of *P. atrus* was largely composed of polychaetes (Annelida).

The dominance of crustaceans in the diet of *H. uarnak* may not necessarily be evidence of selectivity for these prey items, since the low abundance of crustaceans measured in the environment (and hence high degree of selectivity for this prey item by *H. uarnak*) could have been an artefact of the use of core sampling. Many crustaceans, including the penaeid prawns and brachyuran crabs that were prevalent

in the diet of this species are epibenthic and very mobile and would be far less likely to be sampled by a core than infauna such as annelids. Additionally, abundances of crustaceans in the guts of *H. uarnak* may be inflated relative to other prey items because of the resistance of their exoskeletons to the process of digestion.

The high degree of overlap in the diets of *P. atrus, N. kuhlii T. lymma* and *U. asperrimus* could have a number of explanations. Dietary overlap might occur if prey were not limiting, or alternatively, if rates of predation kept abundances of rays at levels below which food became limiting (Vaudo & Heithaus 2011). Large sharks that feed on rays such as the great hammerhead, *Sphyrna mokarran* (Rüppell 1837) and tiger *Galeocerdo cuvier* (Péron & Lesueur 1822) are common at Ningaloo Reef (Stevens *et al.* 2009) and despite the abundance and diversity of rays that are found within this habitat, it seems plausible that these apex predators could maintain ray populations below levels at which competitive exclusion might occur. Additionally, most of the rays were collected during August–December when productivity of soft-sediment habitats peaks (O'Shea *et al.* Accepted). If these conditions provided super-abundant prey, then dietary overlap among species might not incur any competitive costs. Since there is some evidence from temperate environments that prey selectivity in elasmobranchs is correlated to seasonal shifts in prey abundance (e.g. Platell *et al.* 1998a; Lucifora *et al.* 2006), future work should compare the diets of rays at Ningaloo throughout the year to account for seasonal variation in infauna abundance.

Dietary overlap among rays could also be indicative of resource partitioning on other ecological axes, such as space. This may be the case for *N. kuhlii* and *T. lymma*, which had virtually identical diets, but were observed to feed in different microhabitats on the reef. The former species was usually found feeding at the edge of the reef while the latter could typically be found feeding in sand patches within the coral reef matrix. Thus, for these species, partitioning may occur on the scale of metres within the reef, allowing diets to overlap. *Pastinachus atrus* also had a diet that was very similar to both *N. kuhlii* and *T. lymma*, but unlike these species, this ray occurred over a wide area of the reef lagoon and was observed feeding along the edges of the reef and throughout the broad sand plains between the shore and the reef crest. The range of *P. atrus* within the lagoon possibly reflects the fact that this species grows to much larger adult sizes (≤ 200 cm W_D) than both *N. kuhlii* and *T. lymma* (~ 50 cm W_D and 35 cm W_D). This trait may provide some defence against predators to which the smaller-bodied rays are susceptible and allow it venture greater distances from the shelter of the reef to feed on prey inaccessible to the other species.

Individuals of *H. uarnak* targeted crustaceans and this species were never seen feeding during daylight hours. Typically, this species was immobile and partially buried in sand at the edge of the reef when it was encountered. While the abundance of crustaceans clearly separated the diet of this ray from that of my other study species, very similar diets occur among other members of the same genus (e.g. *H. fai, H. toshi and H. astra*; Vaudo & Heithaus 2011). It is unclear how food resources

are partitioned among these cogeneric species, since at least four co-occur at Ningaloo Reef (Stevens *et al*. 2009). This will require sampling that targets the remaining species in this genus.

Similar to my study, earlier work in both tropical and temperate environments has also found that different species of rays within a habitat tend to occupy equivalent trophic roles, feeding on the same types of prey, albeit with some differences in the proportions of prey in the diet among species (Sommerville *et al*. 2011; Vaudo & Heithaus 2011; Yick *et al*. 2011). Patterns of prey consumption probably occur over wide areas of coast, since the same patterns of diet preferences were recorded for the two species (*P. atrus* and *H. uarnak*) ~ 300 km south at Shark Bay (Vaudo & Heithaus 2011). This implies that the trophic roles of these mesopredators may be consistent across broad (100s—1000s km) spatial scales.

There was some evidence for ontogenetic changes in the diets of the one species for which analysis was possible (*H. uarnak*) although my results must be treated with caution due to sample sizes that may have not been sufficient to completely characterise diets of juveniles. Dietary shifts among ontogenetic stages are common in many (Wetherbee & Cortés 2004) but not all elasmobranchs (Clarke *et al.* 1996; Scharf *et al.* 2000; White *et al.* 2004; Lucifora *et al.* 2006; Marshall *et al.* 2008) and are thought to occur for a number of reasons, including increased metabolic requirements as animals mature, greater prey-handling ability as animals increase in size (White *et al.* 2004) and an increase in the habitat available for foraging as animals get larger, allowing access to a wider range of food resources (Wetherbee &

Cortés 2004). Recent tracking studies within the lagoon at Ningaloo have demonstrated that juveniles of my study species may reside in shallow (2—3 m water depth) embayments at the edge of the shore for protracted periods (up to 18 months; Cerutti-Pereyra *unpub. data*). Although adult rays are also found within these habitats, they tend to range over much greater areas of the lagoon than juveniles (Cerutti-Pereyra *unpub. data*), and thus these differences in diets may reflect the wider range of foraging habitats available to adults than juveniles.

Sex-based differences in diet were only evident for *N. kuhlii*, with the females of this species consuming slightly higher proportions of annelids and other prawns than males. Where sample sizes allowed analysis, no evidence for differences in diet of males and females in the remaining species was detected, which is similar to many other studies of rays (e.g. White & Potter, 2004; Navia *et al.* 2007; Jacobsen & Bennett 2012; Lipej *et al.* 2012). However, in other elasmobranchs, partitioning of trophic resources according to sex can be commonplace and is thought to occur due to spatial patterns of sexual segregation within a population where males and females occupy different habitats (Springer 1967) or due to differences in size between sexes (Simpfendorfer *et al.* 2001).

In conclusion, dietary overlap was common among rays at Ningaloo Reef, with annelids dominating the diets of the majority of species. However, one species had low dietary overlap with a diet dominated by crustaceans. Of the remaining species, small-scale (m—100s m) spatial partitioning may allow these animals to consume similar diets. Alternatively (or in addition) food resources may not be limiting in this

environment, either due to the abundance of invertebrate infaunal communities or to predators keeping ray populations at levels below those where they deplete these resources.

Chapter 5:

Bioturbation by Stingrays

Introduction

In the vast soft-sediment environments of the oceans, great numbers of species are bioturbators, creating, shaping and modifying the physical and biological properties of this habitat. Typically, this is done through behaviours such as feeding, digging or burrow formation by animals such as crabs (Eggleston *et al.* 1992), worms (Mermillod-Blondin & Lemoine 2010), sea cucumbers (Shiell & Knott 2010), urchins (Needham *et al.* 2010), dugongs (Nakaoka *et al.* 2002), turtles (Lazar *et al.* 2010), teleosts (Hall *et al.* 1990), elasmobranchs (Valentine *et al.* 1994) and even whales (Oliver & Slattery 1985).

Of the elasmobranchs, rays are one of the most obvious and ubiquitous fishes that act as bioturbators. This diverse group of cartilaginous fishes (over 600 living species) occupies marine ecosystems from the Arctic to the tropics (McEachran & Dunn 1998; McEachran & Fechhelm 1998; Frisk 2010). In shallow coastal and nearshore environments the Dasyatidae, or stingrays, are abundant and inhabit soft-sediment habitats ranging from mangroves to sandy shores and coral reefs (Snelson Jr *et al.* 1988; Gilliam & Sullivan 1993; Cartamil *et al.* 2003). The dorso-ventral compression of rays is thought to assist these animals to exploit shallow tidal areas for prey (Matern *et al.* 2000) where they feed by jetting water and beating pectoral fins to access infaunal and meiofaunal communities in soft sediment, a process of bioturbation that typically produces conspicuous feeding pits.

Chapter 5: Bioturbation

Rays frequently occur in large schools when feeding and migrating (Peterson *et al.* 2001), consequently they have the potential to exert a significant impact on both the physical environment and biological communities that inhabit soft-sediment habitats. Feeding activity by rays on intertidal and subtidal sediments can significantly reduce benthic populations of harpacticoid copepods (Reidenauer & Thistle 1981), polychaetes and bivalves (Pridmore *et al.* 1990). Furthermore, rays have been implicated in severe damage to commercial shellfish operations (Smith & Merriner 1985; Blaylock 1989; Myers *et al.* 2007), as well as destroying seagrass beds (Orth 1975; Hovel & Lipcius 2001; Collins *et al.* 2007).

Despite the abundance and diversity of rays in both tropical and temperate shelf environments and their effects on benthic assemblages, there have been relatively few attempts to quantify patterns of bioturbation by these animals. Here, I quantify bioturbation by rays on soft sediments of a lagoon at Ningaloo Reef in Western Australia. Given that stingrays move in and out of shallow tidal areas on the reef in daily cycles (Cerutti-Pereya *unpub. data*), I hypothesised that new pits would form after each high tide that allowed access to feeding areas. If rays are highly efficient feeders, it would be expected that there should be little evidence for re-use of feeding pits and that pits should infill at relatively constant rates. I tested this hypothesis by surveying fixed quadrats in a feeding habitat and monitoring rates of infill of pits. Finally, given that large numbers of rays can inhabit shallow coastal zones, I examined the amount of sediment turned over by bioturbation by rays in a primary feeding habitat. In order to give a broader context to the impact of rays on sediments,

I surveyed ray pit formation over a large area of lagoon and reef (1 km^2) that contained a variety of inter-reefal habitats.

Material and Methods

Study Locations

This study was conducted at two locations within the Ningaloo Reef Marine Park, Western Australia; Mangrove Bay (-21.9762, 113.9598) in the north and Coral Bay (-23.1335, 113.7703) in the lower section of the marine park (Figure 5.1). A marine protected area (sanctuary zone) in which all fishing is prohibited is in place at Mangrove Bay and extends for approximately 3 km from the shoreline to the outer reef and runs 4 km from north to south along the shore. Tidal range of Mangrove Bay during sampling was ≤ 1 m and the maximum water depth where pits were surveyed was 1.3 m. The sanctuary zone encompasses a small area of mangroves that are unique in this environment since they are found in very few other places within the Marine Park. My study site was the intertidal zone immediately adjacent to mangroves in the southern half of the bay. Within the bay, a large sand spit acts a tidal barrier forcing flooding tides round its head and then into the southern portion of the bay. Between the spit and the beach an area of approximately 100,000m^2 of muddy sands are exposed at low tide. The northern half of the intertidal zone of the bay consists of low-profile limestone reef with abundant macroalgae and very little sand.

At Coral Bay, I sampled an area of approximately1 km^2 (1,000,000 m^2) immediately south of the main boat launching facility, extending to the southern sanctuary zone

marker. The lagoon within this area was dominated by expanses of sand bordered on the seaward side by reef with high coral cover, which sheltered the lagoon from current and swell. Maximum tidal range at Coral Bay was ≤ 1 m and the maximum depth of the Coral Bay lagoon sampled was 10 m.

Sampling

At Mangrove Bay, a total of 15 quadrats of 100 m² were monitored for seven days in each of November 2009, September 2010 and February 2011. Pits were measured after the first high tide each day in order to estimate rates of formation/infill every 24 hours. Quadrats were haphazardly placed in the area of muddy sediments to the south of the bay and within each quadrat, pits were identified, marked with a tent peg and high-visibility ribbon and positions recorded using a GPS. There are many bioturbating organisms that share this environment, so only pits that could be attributable to rays were included in the sampling and any depression or excavation which could not be unambiguously identified as due to a stingray was not included (Figure 5.2A).

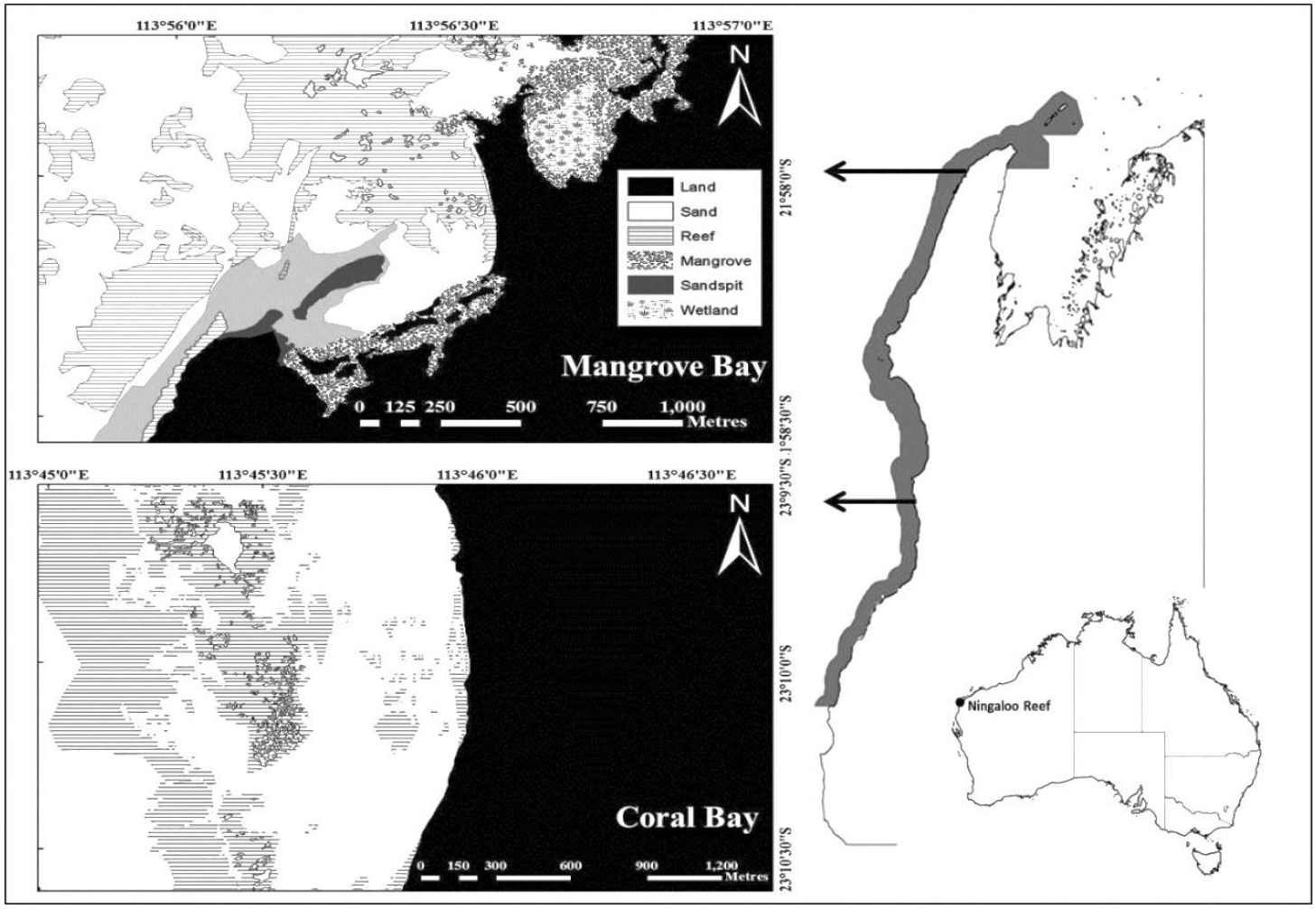


Figure 5.1: Ningaloo Reef Marine Park and the two study locations, Mangrove Bay and Coral Bay

The length, breadth and depth of pits was measured daily for a week or until the pits could no longer be discerned from surrounding sediment in order to test hypotheses relating to infill of pits and re-use by rays. Measurements were made using a tape measure and precision of measurements was approximately ± 1cm for each pit. Any new pits were also marked and measured. All pits were examined for any secondary use by other fauna, such as crabs or juvenile fish (Figure 5.2B).

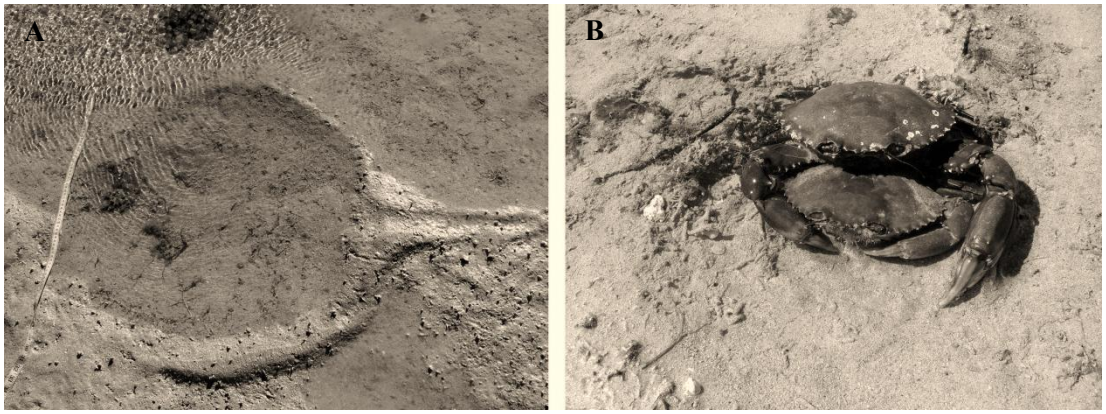


Figure 5.2: (**A**) New pit created by feeding ray, and (**B**) degraded pit with *Scylla serrata* occupying excavation

In Coral Bay, the lagoon habitat was surveyed for the presence of rays and pits in depths between 2 and 9 m. This was done in order to give a broader context to the impact of rays on sediments across a variety of soft-sediment habitats within the lagoon. Lagoon habitats were mapped and rays and pits recorded three times over this area in August 2009, August 2010 and February 2011. Observations were made by two snorkelers towed at 15 and 25 m behind a boat using manta boards (methods described by Miller & Müller 1999). Up to 15 – 20 transects spaced between 30 – 60 m apart were required to survey the entire area of 1 km^2. The variation in numbers of

transects were related to weather conditions and visibility. When there was lower visibility, transects were spaced closer than in good conditions, in order to ensure that observers covered the entire sampling area. The first observer would record the habitat immediately beneath them every 10 s in one of five categories (sand, coral reef, biogenic rubble, turf algae and seagrass), while the second would record ray pits and the presence of rays. The position of pits and rays were recorded using a GPS. The observer also recorded species identity and approximate size of all rays.

Analytical Procedures

To test the hypothesis regarding quantities of sediments being displaced by rays during activity, at Mangrove Bay I calculated the volume of empty pits by treating each (n = 108) as a semi-ellipsoid, using the equation:

$\frac{4}{3}\pi$ (Lr × Br × Dr)/2;

where Lr = length radius, Br = breadth radius and Dr = depth radius. Wet weights of sediment for these volumes were extrapolated using the mean weight of 10, 1-cm³ samples of wet sediment from the same site. The intensity of disturbance created by rays feeding over the entire bay was determined by summing total pit area and dividing by the total sampling area to give a percentage of the total area disturbed. In order to determine the how long the pits persisted in the sediment at Mangrove Bay over the course of the seven day sampling period I fitted a generalised linear mixed model (GLMM) using a binomial distribution and a logit link function where the response variable was presence/absence of pits and the fixed, explanatory variable was time (day of the sampling period). I therefore modelled the probability of a pit

being present as a function of time. As individual pits were sampled repeatedly over time, individual pits were coded as a random effect to account for the temporal dependence structure between the observations. Pits were sampled over three years, thus pits were nested in years. Models were fit using the lme4 library in R: a Language and Environment for Statistical Computing (R development core team 2011) where the random effect was the individual pit nested in year. I used an information-theoretic approach to test for an effect of time by comparing Akaike's information criterion corrected for small samples (AIC_c) (Burnham & Anderson 2002) and the AIC_c weight ($wAIC_c$) of the slope model (probability of a pit being present ~ time + year/pit) to the intercept-only (null) model (probability of a pit being present ~ 1 + year/pit). The intercept-only model (or null model) is a model that does not contain any β (effects), except for an intercept. In this way I compared two models that were the same, except that one had the effect of interest (time) and one does not. The $wAIC_c$ is a measure of the models relative goodness of fit and varies from 0 (no support) to 1 (complete support) (Burnham & Anderson 2002).

Changes in the volume of pits over the sampling period were also examined using a linear mixed-effects approach. This analysis aimed to determine if rays were re-using a previously excavated pit, which would have created an increase in the volume of the pit over time, or pit volumes remaining static over time. All pits that were present for less than three days were removed from the analysis, as I could not fit a line to only two points. Pit volume was modelled as a function of day with the random effect pit nested in year and this model was compared to the null model as described above. Data were log transformed and the models fitted using the R library nlme.

Results

Mangrove Bay

A total of 108 pits were sampled over 21 days, equating to 2.42% of the area sampled and 0.031% of the entire soft-sediment habitat of the Mangrove Bay intertidal zone (Figure 5.3). The sediments excavated by rays during this time equated to 1.08 m³ with a wet weight of 760.8 kg, and the mean volume of pits from all years was 10,064 cm³ (± 1,487 SE). The numbers of pits varied among the three sampling times, but most notably in November 2009, when only 19 pits were found, accounting for 17.6% of the total number of pits found over the three sampling periods. In comparison, counts of pits in September 2010 and February 2011 accounted for 42% (n = 45) and 40% (n = 44) of total numbers respectively.

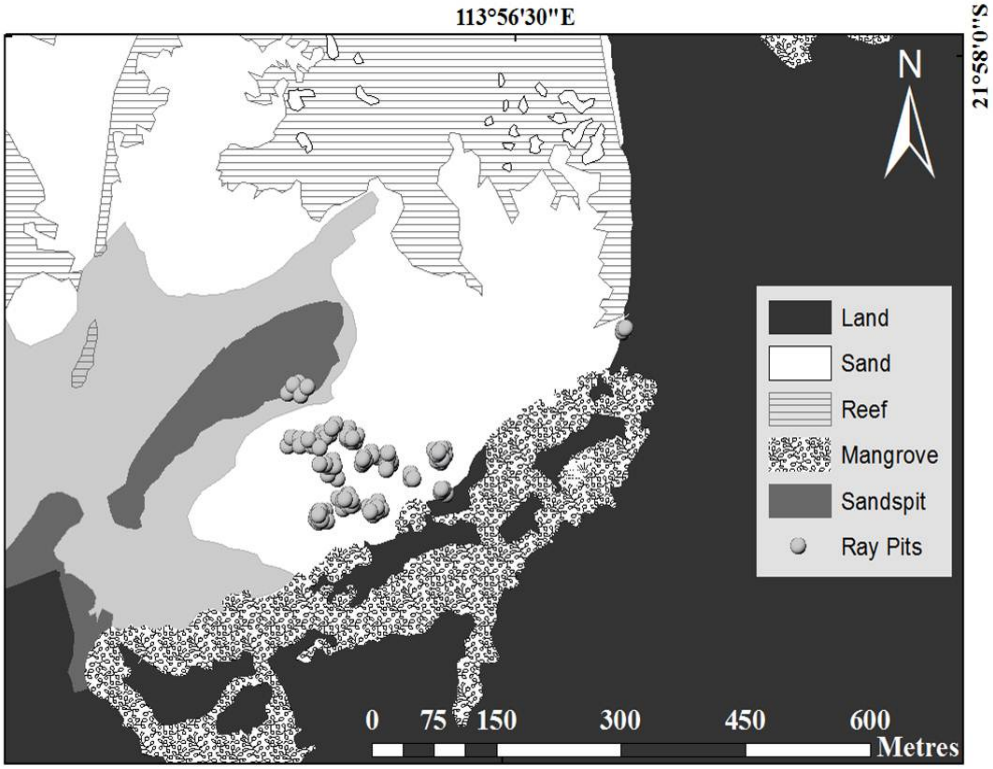


Figure 5.3: Mangrove Bay southern intertidal zone and position of all sampled pits

Pits ranged in volume from 334 cm³ - 100,577 cm³. Approximately 80% were relatively small (Supp. Figure 5.1 - Appendix 2). The estimated volume of pits also varied among sampling times, with mean volume in 2009 (21,939 cm^3 ± 4,774 SE) almost two and half times greater than in 2010 (8,782 cm^3 ± 2,507 SE) and three and half times greater than in 2011 (6,302 cm^3 ± 1,115 SE).

Longevity

The probability of a pit being present declined over the seven-day sampling period as indicated by 100% support for the model that included day as a factor (*w*AIC = 1) (Table 5.1). There was an 80% probability of an average pit being present on day one, with the probability of presence then rapidly declining to a low of 45% around day 4, to a low of 15% after seven days (Figure 5.4).

Table 5.1. Ranked general linear mixed effects models of the probability of a ray pit being present explained by day and random effects (pit nested in year), and the volume of ray pits explained by day and random effects (pit nested in year) LL, maximum log-liklihood; *k,* number of estimate model parameters; AIC_c, Akaike's Information Criterion for small samples; Δ AIC_c, change in AIC_c relative to the to ranked model ; *W*AIC_c, AIC_c weight

Model	LL	*k*	AIC_c	ΔAIC_c	*w*AIC_c
Presence ~ day + (1year/pit)	-398.80	4	805.65	0	1
Presence ~ 1 + (1year/pit)	-460.94	3	927.92	122.27	0
Volume ~ 1 + (1year/pit)	-303.89	4	615.84	0	0.91
Volume ~ day + (1year/pit)	-305.20	3	620.51	4.66	0.09

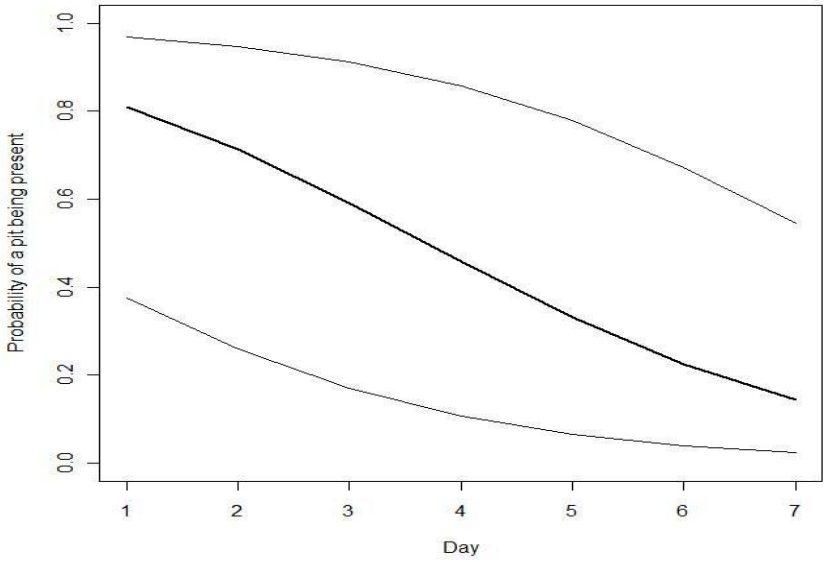


Figure 5.4: GLMM predicted probabilities of pit presence over time. The thick line in the middle represents the predicted probabilities for all pits and the lighter lines either side are 95% confidence intervals

Secondary use

There was little evidence for a relationship between volume of the pit and day of sampling (Supp. Figure 5.2 - Appendix 2) as the intercept only model (null) had majority support (91%) (Table 5.1). Thus, on average, pit volumes remained static over the sampling time, suggesting that re-use of pits by rays or other species was occurring. As the binomial model suggested that probability of a pit being present declined over the sampling period, it would have been reasonable to expect that the model for volume should also show a declining relationship. This did not occur, probably due to the inconsistent nature of the relationship between volume and day among years and pits, as can be seen in the individual plots of pits per year (Figure 5.5 and Supp. Figures 5.3 and 5.4 – Appendix 2). Overall, a decline between volume and time occurred in 48% of pits, with the remaining pits showing a

static (22%) or increasing (30%) volume. In 2009, 35% of pits increased in volume over 7 d (n = 17) (Figure 5.5); in 2010, 46% of pits increased in volume, (n = 11) (Supp. Figure 5.3 - Appendix 2) and in 2011, 17% of pits increased in volume (n = 18) (Supp. Figure 5.4 - Appendix 2). Of the 22% of pits that did not change in volume throughout the period of sampling, 12% occurred in 2009 (Figure 5.5); 36% in 2010 (Supp. Figure 5.3 - Appendix 2) and 27% in 2011 (Supp. Figure 5.4 - Appendix 2). These results are evidence for re-use of the pits either by rays or other organisms.

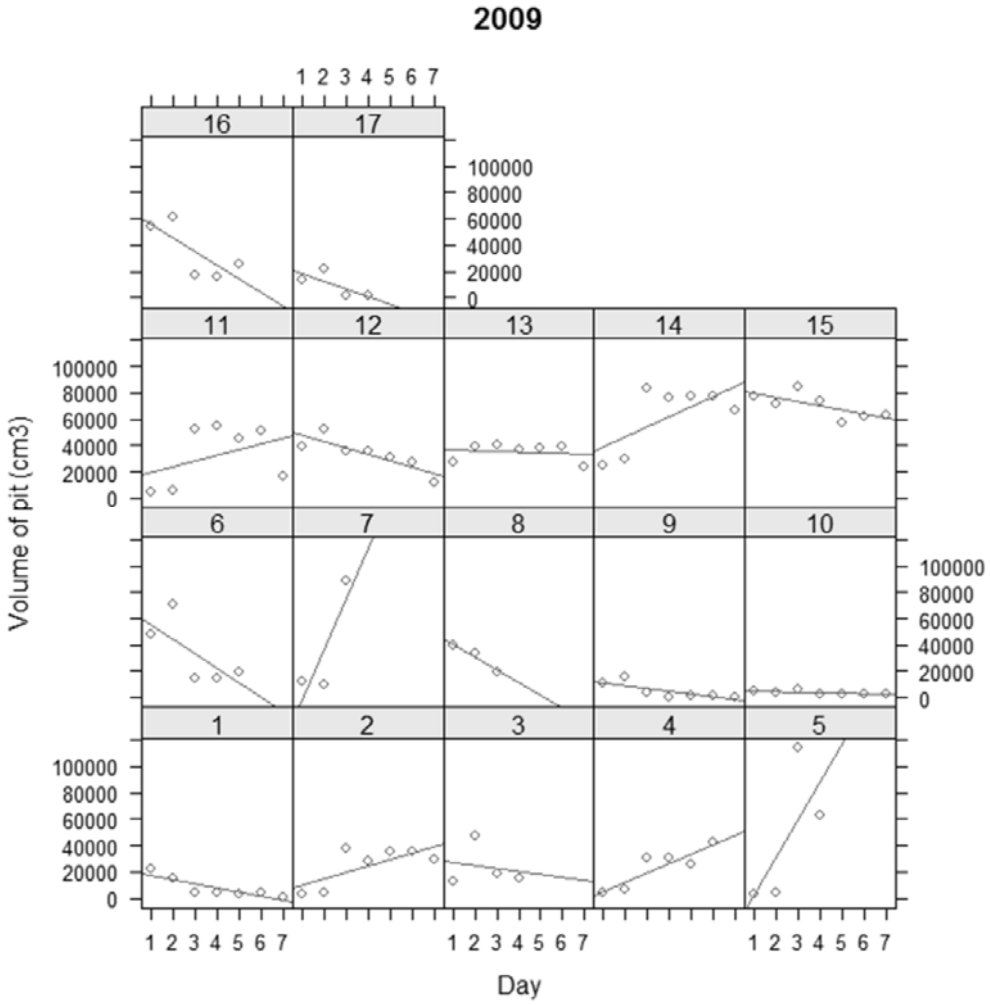


Figure 5.5: Linear relationships between day of sampling and volume of each of the pits sampled from November 2009 that were present for three days or more

Rays were not directly observed re-using pits; however observations did indicate secondary use by other taxa in all new pits formed during the study. Small fish were the most common occupants at low tide (90% of all pits), where pits remained full of water. Adult fish were occasionally seen in larger pits (> 10,000 cm³, n = 7); however, over half of these were dead or dying, probably as a result of attacks by sea birds. Invertebrates such as gastropods (*Nerita* sp.) were found in 87% of all pits.

Accumulation of detrital material and seaweed was common in every pit examined at low tide, and this in turn created a potential refuge for organisms such as crabs. The mud crab *Scylla serrata* was abundant at Mangrove Bay and 25% of pits had at least one adult inhabiting it on day 1 when sampling began; while newly formed pits were occupied by this species in 10% of cases within 24 hours.

Coral Bay

The lagoon at Coral Bay was dominated by sand (48%) and coral reef (36%). Biogenic rubble (6%) and turf algae (10%) were present in all years; however seagrass (< 1%) was only documented in February 2011 at the far southern end of the map boundary. Over the three sampling periods, a total of 20 rays from six species and 37 ray pits were observed, with the highest number of both rays (n = 9) and pits (n = 14) recorded in the first sampling session during August 2009. A total of 11 pits and 6 rays were recorded in August 2010 and 12 pits and 5 rays recorded in February 2011. Of the 37 pits, 92% were recorded in sand and 8% were recorded in sand where turf algae was also present. Six species of ray were sighted during the sampling: *Urogymnus asperrimus* (n = 5), *Neotrygon kuhlii* (n = 4), *Taeniura lymma* (n = 1), *Himantura uarnak* (n = 3), *Pastinachus atrus* (n = 6) and *Taeniura meyeni* (n = 1). Over half (55%) of all rays were buried in sand and of these, all were found within 2 m of coral, or some form of structure. *T. lymma, N.kuhlii* and *U. asperrimus* were all found immediately adjacent to reef, buried and inactive. *T.meyeni* was observed swimming in mid-water, as were two *P. atrus*. All *H. uarnak* and the remaining *P.atrus* were found feeding or resting in open sandy habitats.

Discussion

Sediment removal

This study shows that rays can be significant agents of bioturbation in the intertidal area of a coral reef ecosystem. Ray feeding pits over seven days disturbed an average of 2.42% of an area of intertidal habitat of 500 m². When extrapolated to a year, this would result in sediment turnover of 42% of the entire intertidal soft-sediment habitat (~ 42,000 m²) to a mean depth of 5.6 cm. This estimate is comparable to an earlier study of bioturbation by rays (*Dasyatis americana, D. sabina* and *Gymnura micrura*) in a temperate estuary in South Carolina, where 30% (6000 m²) of the study area was covered in ray pits during a July sampling period (Grant 1981). Larger volumes of sediment were reportedly re-worked by *Myliobatis californica* and *Urolophus halleri* at Bahia La Choya in Mexico (Myrick & Flessa 1996). Their study found that these two species of ray were overturning sediments at an average rate of 1.01 m^3/m^2/year, with > 100 new pits formed every 24 hours. In comparison, rays at Mangrove Bay overturned sediments at the much lower rate of 0.167m^3/m^2/year.

Comparable Taxa

It is difficult to compare estimates of bioturbation by rays with those of other large vertebrates in coral reef systems because very few studies exist. The potential for bioturbation by animals such as the dugong (*Dugong dugon*) is well recognised, with numerous studies of the frequency and effects of feeding scars on benthic habitats and biological communities (Heinsohn & Birch 1972; Nakaoka *et al.* 2002; Skilleter *et al.* 2007). However, there has been no attempt to quantify the volume of material or turnover rates of sediment moved by these animals. In contrast, bioturbation by

invertebrate taxa, notably callianassid shrimps, has been documented extensively in coral reef systems (Branch & Pringle 1987; Murphy & Kremer 1992; Tudhope & Scoffin 1984). These shrimps are deep burrowers and the volume of sediment that they are capable of processing is immense. Myrick and Flessa (1996) estimated that these shrimps turned over sediment on a sand flat in Mexico at an average of 0.56 m^3 /m^2 /year; a rate 3.5 times greater than the 0.167 m^3 /m^2 /year I recorded for rays at Mangrove Bay. Similarly, Riddle (1988) found that the physical effects of powerful cyclones on soft sediments were quickly erased (within 6 weeks) by the action of callianassid shrimps in the lagoons on the Great Barrier Reef.

Mangrove Bay vs. Coral Bay

This study examined an intertidal sandflat that was adjacent to an important habitat for juvenile rays and other elasmobranchs. Acoustic tracking studies have shown that rays, particularly juveniles and adult females are present in this subtidal habitat year-round and that this area may function as a nursery for a variety of ray species (Cerutti-Pereya *unpub. data*). Thus, the rates of bioturbation I recorded in this area may not be representative of the wider lagoon of Ningaloo Reef. My surveys of the southern lagoon suggest that this is the case. Sampling over 1,500 m^2 of the sandflat at Mangrove Bay recorded 108 feeding pits, while manta tows over 1 km^2 of the lagoon at Coral Bay recorded only 37 pits during three surveys. However, it is likely that the greater current flows and 'clean' sand in the lagoon at Coral Bay result in much faster disintegration of feeding pits at this locality than at Mangrove Bay. A total of six species of ray were sighted by my surveys at Coral Bay. These species

are common throughout Ningaloo Reef (Stevens *et al.* 2009) and it is likely that they are responsible for creating the feeding pits at Mangrove Bay. At high tide these rays move into the intertidal, presumably to feed although these shallow waters may also provide a refuge from predation. It is probable that I under-estimated numbers of rays in the lagoon of Coral Bay, since the smaller species tend to be cryptic, either burying themselves in sediment (e.g. *Neotrygon kuhlii*) or hiding under reef outcrops (e.g. *Taeniura lymma*).

Longevity

The longevity of ray pits has received little attention, despite the possibility that they create micro-habitats that may differ from surrounding areas in carbon transport, nutrient regeneration, sediment stability and decomposition processes (Austen *et al.* 1999). The formation of ray feeding pits may create bio-geochemical gradients that in low-energy environments may take many days or weeks to infill, which may account for some of the unexplained variation in the structure and abundance of benthic communities on smaller (cm – m) spatial scales (Zajac *et al.* 2003). As expected, I found a negative relationship between pit presence and time, as pits were not permanent structures and were subject to in filling. These model results showed that there was a reduced probability of an average pit remaining after 4 days (~ 40%) and at the end of the 7-day sampling period there was on average only a 15% probability of a pit still being present. My analysis of the change in pit volume over time, however, did not always follow the same negative trend. Over the seven days of monitoring, only 48% of pits in-filled while the rest remained static or increased in volume. This latter result is

evidence for reuse of pits by other taxa, some of which are known bioturbators, such as crabs. The slow disintegration of pits at Mangrove Bay was also probably related to the protection of the habitat from strong wave action and current flow. Given that they are common and relatively persistent structures in the soft sediment, it is likely that pits play an important role in shaping population distributions and structures of infaunal communities (Zajac *et al.* 2003). It has been proposed that losses of bioturbating organisms could impair marine ecosystem function (Thrush & Dayton 2002; Lohrer *et al.* 2004) and yet despite this, few data are available on pit formation by rays. Biological effects of ray pits are well documented (e.g. VanBlaricom 1982, Cross & Curran 2000) and typically demonstrate that infaunal communities are removed by ray feeding, which is followed by a rapid re-colonisation of pits by ostracods and amphipods (VanBlaricom 1982). On a microbial level, the creation of pits can allow oxygen to penetrate deeper into sediments, extending the zone of nitrification (Gilbert *et al.* 1995) and even affecting the nitrogen cycle compromising functions of specific bacterial groups (Kogure & Wada 2005). These responses highlight the importance of pit formation in the ecology of marine soft sediment environments and any loss of rays in these habitats may lead to changes in lower trophic and biogeochemical levels.

In conclusion, this study has quantified the persistence of ray pits, rates of infilling and sediment turnover rates in an intertidal area of a coral reef ecosystem. In doing so, I have demonstrated that bioturbation by rays can be a significant functional process in coastal and nearshore environments and may be critical to physical,

biological and chemical processes at least in some intertidal habitats. Despite 42% of the soft sediment habitat of Mangrove Bay being re-worked on an annual basis, this rate of turnover was much lower than recorded by earlier studies of bioturbators, which have tended to focus on temperate marine ecosystems. Furthermore, bioturbation may be relatively trivial when considered in the context of sediment turnover by tidal and wave action (Grant 1981). This implies that ray pit formation might be most relevant to biological communities on micro (cm) and meso (10s m) rather than meta (100s m – km) scales (Zajac 2004). Future work will examine the prey and selectivity of ray feeding at Ningaloo Reef and its effects on infaunal communities.

Chapter 6:

Experimental manipulation of stingray foraging in a tropical intertidal bay at Ningaloo Reef, Western Australia

Introduction

Rays are a ubiquitous group of excavating bioturbators that occur in nearshore waters worldwide. They displace large volumes of sediment during feeding, creating conspicuous pits in soft sediment habitats (O'Shea *et al.* 2012). This behaviour has significant consequences in these environments by altering the physical environment (O'Shea *et al.* 2012) and composition of benthic communities (VanBlaricom 1982). For example, foraging by stingrays has been shown to contribute to the destruction of seagrass beds and commercial fisheries for shellfish (Smith 1985; Blaylock 1989; O'Shea *et al.* 2012) and can indirectly influence the abundance and composition of meiofaunal communities as a result of these disturbances (Coull 1990).

Despite the fact that rays are often very abundant in coastal habitats and nearshore environments, much of what is known about the effects of their feeding on benthic communities comes from observation rather than experiments. To date, the most comprehensive experimental assessment has been by VanBlaricom (1982) who manipulated densities of benthic predators including two species of ray (*Urolophus halleri* and *Myliobatis californica*) using caged exclusion experiments. His work demonstrated that while ray feeding initially removed virtually all infauna, patterns of succession included a rapid recolonisation of pits by amphipods and ostracods. While this study clearly demonstrated a wider biological effect attributable to ray

foraging, it is limited in that only two fully enclosed cages were used and the experiment ran for only six months. Merkel (1990) used fences, stakes and erosion mats to exclude the round stingray, *Urolophus halleri* and quantify the rates at which newly transplanted eelgrass survived in habitats where these rays were the dominant benthic predator. Results indicated survival rates during a 23 day sampling period were higher in treatments when compared to controls. Similarly, Fonseca *et al.* (1998) recorded 100% loss of seagrasses within 24 hours of replanting where exclusion cages were not used. Furthermore, Hines & colleagues (1997) suggested density-dependent predation by eagle rays (*Myliobatis tenuicaudatus*) whereby specific patches of benthic habitat are targeted based on prey thresholds (in this case the bivalve *Macomona lilliana*). This suggest further consideration to foraging based disturbances by rays is likely to be dependent on a variety of ecosystem characteristics (Thrush 1999) which also need to be considered when assessing the physical impact of epibenthic predation.

Together, these studies show that rays are clearly agents of disturbance in benthic environments; however they have examined only a limited number of species in a very restricted number of habitats. Very little in known about the effects of feeding by rays in tropical systems, despite the diversity and abundance of rays in these environments. Here, I examine the impact of stingray foraging on infaunal communities in a tropical intertidal embayment at Ningaloo Reef, Western Australia. Rays are common in this habitat and are known to have a major effect on turnover rates of sediment in some habitats (O'Shea *et al.* 2012). I use exclusion experiments to quantify the effects of stingray feeding on infauna of soft sediment habitats and hypothesised that abundances of infaunal taxa between treatment and controls would

change over time, favouring treatments, as stingrays would have a direct impact outside of exclusion zones.

Methods

Study Location and Species

This study was conducted at Mangrove Bay, a sanctuary zone within the Ningaloo Reef Marine Park in Western Australia (S21°58.385' E113°56.99') (Figure 6.1). It is a mangrove-fringed embayment with a large intertidal zone and a mixture of limestone pavement and muddy, fine-grained sediments. A large sand spit is present in the bay and for most of the time (outside of spring tides) it acts as a barrier, separating the lagoon to the west and a sheltered area (~ 100,000 m^2) to the east, predominantly consisting of muddy sands that are immediately adjacent to the mangroves. Throughout the year turf algae is present at varying densities and 50 preliminary sediment cores were taken to test for suitable sites and anoxic layer depth which occurs at approximately 10 cm. Tidal range during the experimental period was ≤ 1 m and maximum depth where the experiment took place was 1.3 m. Previous studies have shown a high number of rays use this bay for feeding (O'Shea *et al.* 2012).

Experimental Design

I constructed ten stingray exclusion zones (4 m^2 each) using steel rods (100 cm length × 1.2 cm diameter) and marine grade rope. Each exclusion zone was constructed using 15 – 20 steel rods hammered into the substrate to a depth of approximately 70 cm, leaving up to 30 cm above the sand, approximately 40 cm apart. Around 30 – 50 m of rope was then wrapped round the rods to create a simple

mesh sitting above the sand surface. The cage-less design of these treatments was to ensure that hydrodynamic processes were not altered and other epibenthic predators were still able to forage, therefore attributing any detectable effects to stingray exclusion.

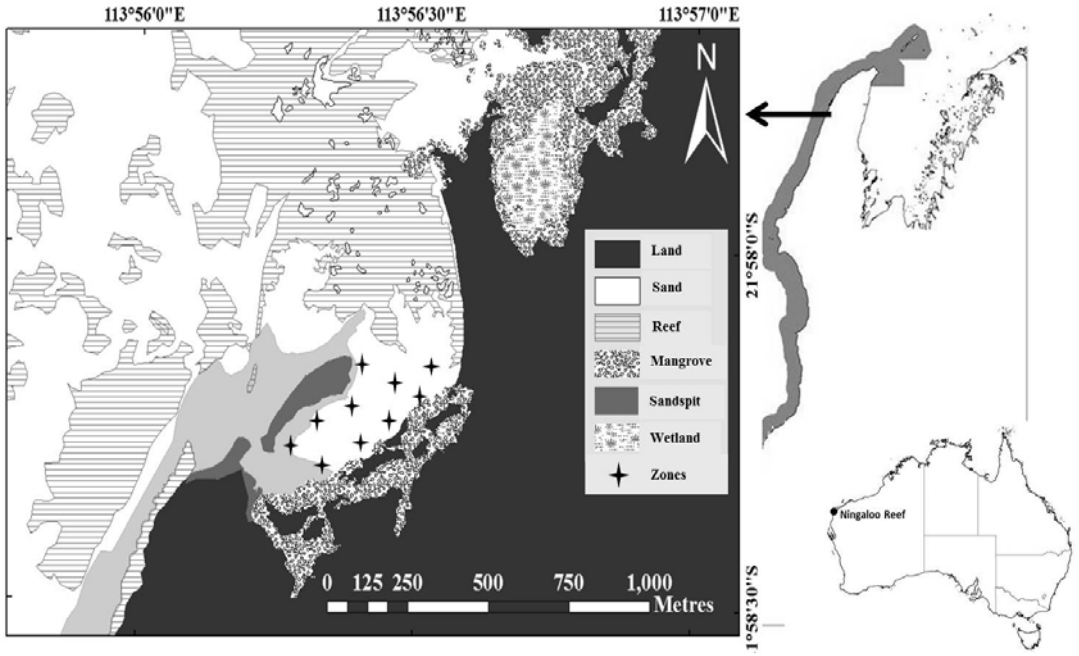


Figure 6.1: Map of Mangrove Bay and treatment locations

The design was based on the assumption that the protruding steel rods would prevent rays from settling to feed if swimming from above and if approaching from the side, the spacing of the rods would also prevent entry. If any ray did manage to enter, then the rope mesh would thwart foraging. To test hypotheses relating to changes in infaunal abundance, I took 10 sediment cores (~ 400 cm^3 each) using 400 ml using plastic sample containers (16 cm x 5 cm x 5 cm) and forcing them into the benthos to a depth of approximately 15 – 18 cm. Five were located inside each ray exclusion zone and another five within a 10 m radius around each exclusion zone where rays

were still able to forage. Sampling took place every 12 weeks from April 2010 to April 2011.

Laboratory Procedures

Samples were stained with Rose Bengal and stored for 24 hours prior to sorting to ensure sufficient staining (after methods outlined in Mason & Yevich 1967). To separate infauna, I washed each sample through a 45-µm sieve using distilled water. All taxa were identified to the highest taxonomic resolution using a dissecting microscope.

Analytical procedures

Analyses were conducted on the top five numerically abundant families, which represented 98% of all taxa sampled. One treatment and one control were removed from the analysis due to a treatment being lost from the study area between time one and time two. To determine whether there were differences between treatments and controls, I conducted a three-way, crossed, non-parametric, univariate, permutational analysis of variance (PerMANOVA) on each of the top five families found in the samples. Count data were square root transformed and resemblance calculated with Euclidean distance. Factors included site (1 – 9; random), test (treatment/control; fixed), and time (1 – 4; fixed). The factor 'time' was treated as fixed to allow for repeated measuring of the exclusion zones through time. If significant interactions were detected from PerMANOVA with the main factor of interest (test), I conducted pairwise comparisons within each two-factor combination to investigate the nature of the effect. All analyses were done in Primer V6 (Clarke & Gorley 2006).

Results

Total sediment volume analysed from all replicate samples taken from treatments and controls (n = 360) equated to ~ 141, 444 cm^3 (141.4 litres). A total of 5,486 individuals from eight families were identified from all experimental samples, made up of 2,858 individuals from the treatments and 2,628 from controls (Table 6.1). The top five numerically abundant taxa were annelids (52%), molluscs (39%), echinoderms (3%), crustaceans (3%) and Platyhelminthes (1%). The three remaining families were chaetognaths, cnidarians and teleosts, all of which combined accounted for 2% (Figure 6.2). While chaetognaths are commonly found in marine benthic habitats, the presence of cnidarians and teleosts were considered opportunistic finds not reflective of infaunal compositions.

Table 6.1: Summary of infaunal taxa collected from all treatments (**T**) and controls (**C**) from each time (1 – 4) to highest taxonomic resolution (HTR)

Phylum	Class	HTR	T1	T2	T3	T4	C1	C2	C3	C4
Annelida; n = 2899			**0**	**6**	**8**	**12**	**0**	**88**	**107**	**138**
		Unidentified Polychaeta	**2**	**4**	**8**	**8**	**1**	**19**	**0**	**2**
		Terebellidae	**36**	**235**	**39**	**29**	**75**	**178**	**24**	**16**
			64	**353**	**320**	**309**	**81**	**307**	**317**	**113**
Chaetognatha; n =13			**4**	**6**	**0**	**0**	**0**	**2**	**0**	**1**
Cnidaria; n = 10	Anthazoa	Cnidaria	**8**	**0**	**0**	**0**	**1**	**0**	**0**	**1**
Chordata; n = 3		Teleostei	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**
Crustacea; n = 142		Unidentified	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**1**
	Malocostraca	Paenaeoidea	**1**	**86**	**1**	**1**	**2**	**21**	**2**	**2**
		(infraorder) Brachyura	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**2**
		Ostracoda	**1**	**5**	**4**	**4**	**0**	**0**	**4**	**0**
Echinodermata; n = 206		Asteroidea	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**1**
		Echinoidea	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**2**
		Ophiuroidea	**0**	**7**	**9**	**7**	**3**	**6**	**1**	**1**
		Clypeastroidea	**11**	**25**	**33**	**12**	**29**	**24**	**20**	**13**
Mollusca; n = 2178	Gastropoda	Unidentified	**72**	**130**	**285**	**229**	**213**	**200**	**234**	**123**
		Cypraeoidae	**5**	**21**	**6**	**6**	**0**	**1**	**3**	**1**
		Cerithiidae	**4**	**28**	**6**	**8**	**0**	**3**	**2**	**4**
		Patellidae	**1**	**5**	**0**	**2**	**0**	**2**	**6**	**3**
		Nassariidae	**0**	**1**	**1**	**5**	**0**	**3**	**2**	**3**
		Trochidae	**1**	**9**	**30**	**30**	**1**	**5**	**1**	**0**
	Bivalvia	Unidentified	**11**	**22**	**83**	**83**	**37**	**48**	**77**	**16**
		Siliqua spp.	**1**	**0**	**0**	**1**	**4**	**1**	**0**	**1**
		Solenidae	**0**	**0**	**2**	**2**	**0**	**0**	**5**	**7**
		Tellinidae	**37**	**37**	**2**	**2**	**0**	**0**	**2**	**2**
Platyhelminthes; n = 35		Unidentified	**0**	**18**	**3**	**3**	**0**	**4**	**1**	**0**
		Cestoidea	**0**	**1**	**0**	**2**	**0**	**0**	**0**	**3**

Univariate analyses for the top five most abundant taxa (Annelida, Mollusca, Crustacea, Echinodermata and Platyhelminthes) indicated non-significant differences between treatments and controls (Table 6.2); however, there was a significant two-way interaction between time and test for Mollusca ($p = 0.004$, $df = 3$) and Platyhelminthes ($p = 0.04$, $df = 3$) and a significant three-way interaction for Echinodermata ($p = 0.05$, $df = 24$) and Crustacea ($p = 0.001$, $df = 24$). Pairwise comparisons within the time × test interaction demonstrated that molluscs were sampled in significantly different abundances between treatment and controls for sampling time one ($p = 0.01$) and four ($p = 0.02$) (Figure 6.3); however the direction of change was different between the two, i.e. there was higher abundance in the control for time 1, whereas there was higher abundance in the treatment for time 4 (Figure 6.4). For crustaceans, there were no significant differences between treatments and controls for any of the times sampled, however time 2 was close to significant ($p = 0.07$) with higher abundance in the treatment compared to control for this sampling time (Figure 6.5). The cause of the significant three-way interaction for Echinodermata and Crustacea can be seen in figures 6.6 and 6.7 – with the direction of change between treatment and control changing inconsistently over sampling times and sites.

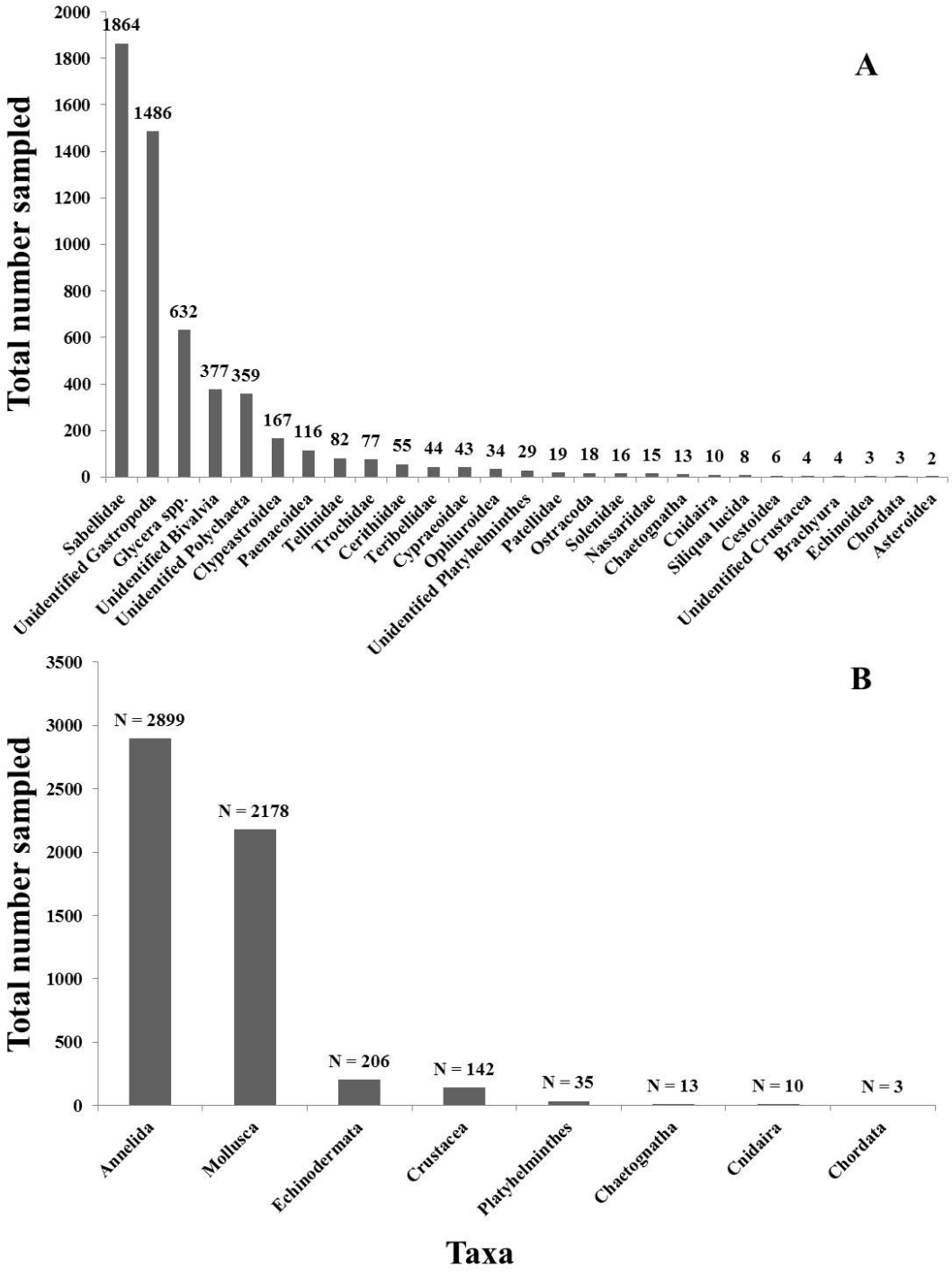


Figure 6.2: (A) Rank abundance of all infauna sampled at the highest taxonomic resolution and **(B)** rank abundance at the family level

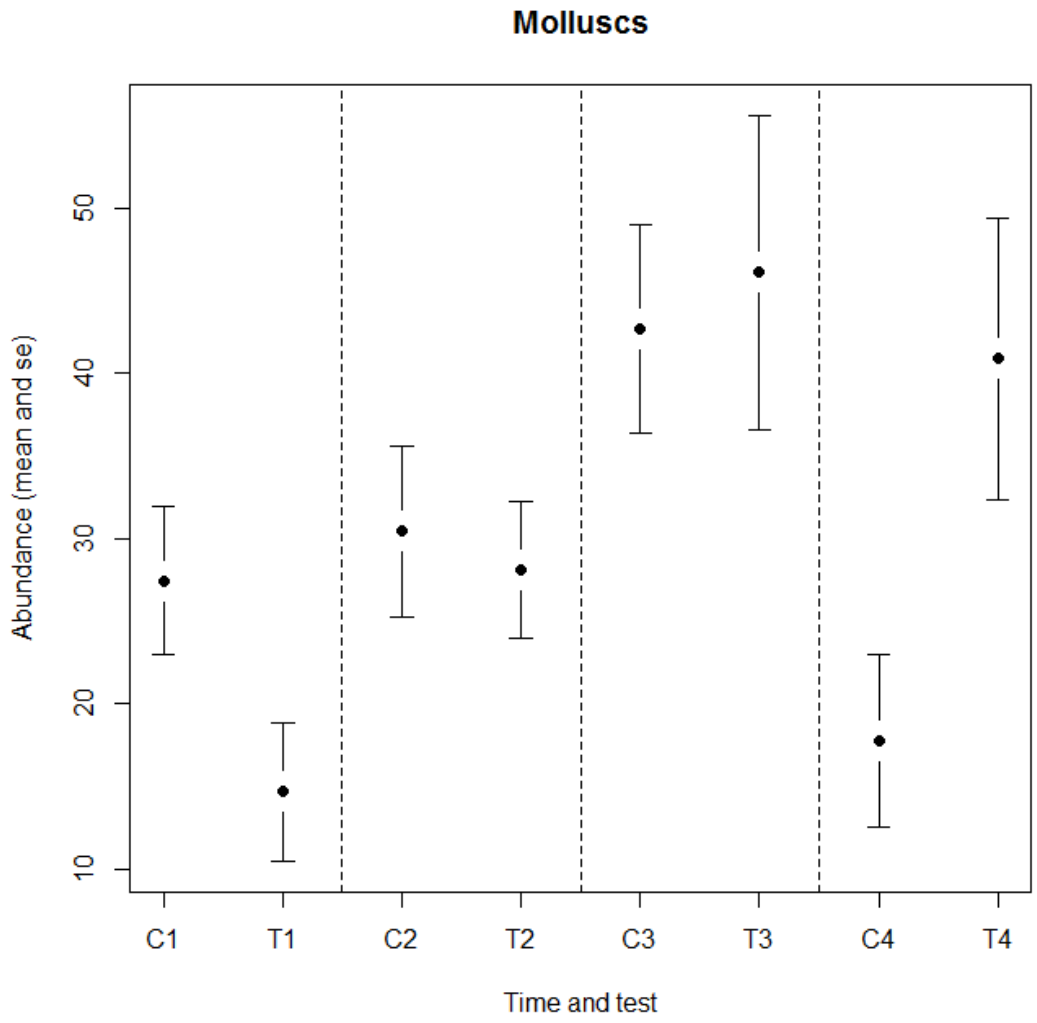


Figure 6.3: Mean abundance of Mollusca between experimental treatments and sampling times (C = controls and T = treatment) and sampling times (1 – 4)

Table 6.2: Results from PerMANOVA main test ((df = degrees of freedom; P (perm) = permutational significance level))

Taxon	Source	df	P
Annelida	Time	3	**0.001**
	Test	1	0.867
	Site	8	0.421
	Time x Test	3	0.134
	Time x Site	24	**0.001**
	Test x Site	8	0.464
	Time x Test x Site	24	0.169
Mollusca	Time	3	**0.002**
	Test	1	1
	Site	8	**0.003**
	Time x Test	3	0.004
	Time x Site	24	**0.001**
	Test x Site	8	0.165
	Time x Test x Site	24	0.227
Echinodermata	Time	3	**0.001**
	Test	1	0.495
	Site	8	**0.027**
	Time x Test	3	0.271
	Time x Site	24	0.433
	Test x Site	8	0.589
	Time x Test x Site	24	**0.048**
Crustacea	Time	3	**0.002**
	Test	1	0.294
	Site	8	0.205
	Time x Test	3	0.057
	Time x Site	24	**0.001**
	Test x Site	8	0.898
	Time x Test x Site	24	**0.001**
Platyhelminthes	Time	3	**0.008**
	Test	1	0.18
	Site	8	0.902
	Time x Test	3	**0.037**
	Time x Site	24	0.256
	Test x Site	8	0.69
	Time x Test x Site	24	0.817

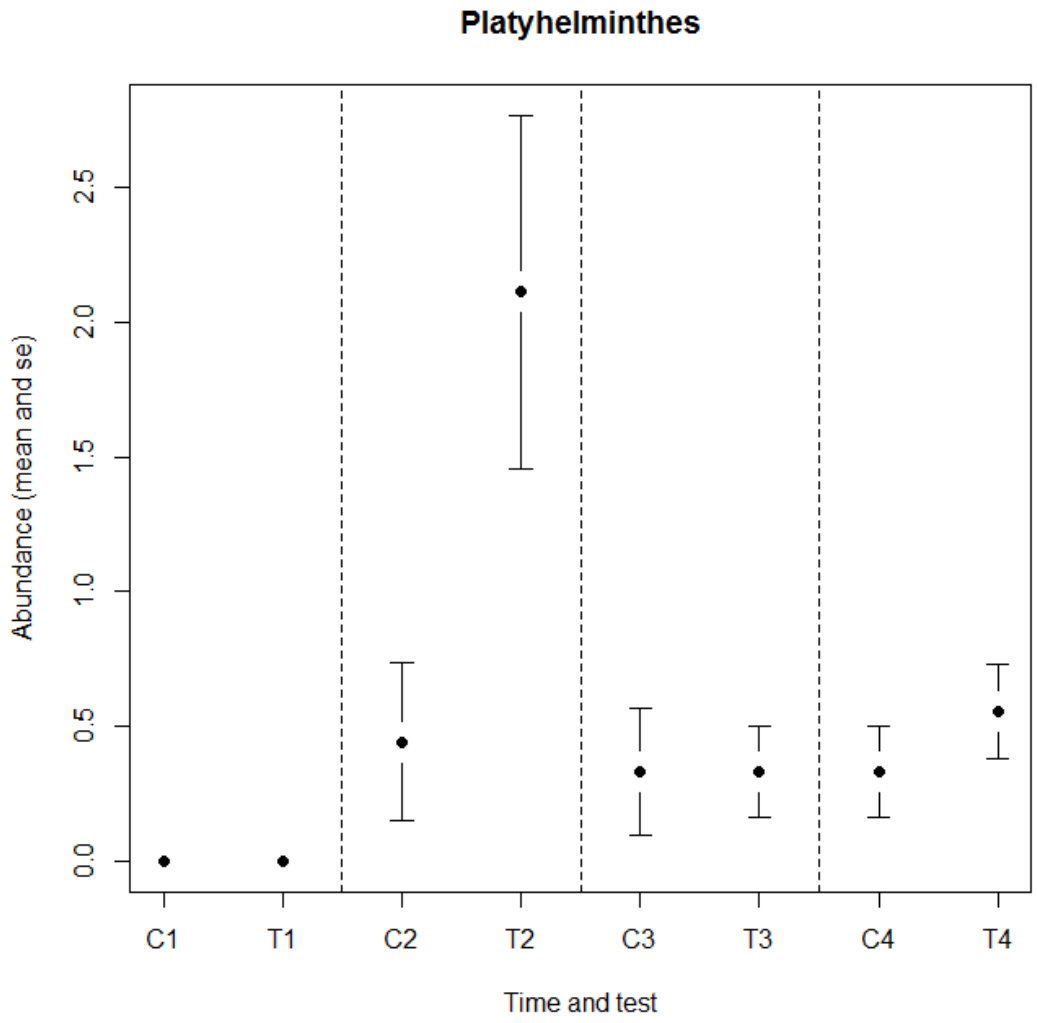


Figure 6.4: Mean abundance of Platyhelminthes between experimental treatments and sampling times (C = controls and T = treatment) and sampling times (1 – 4)

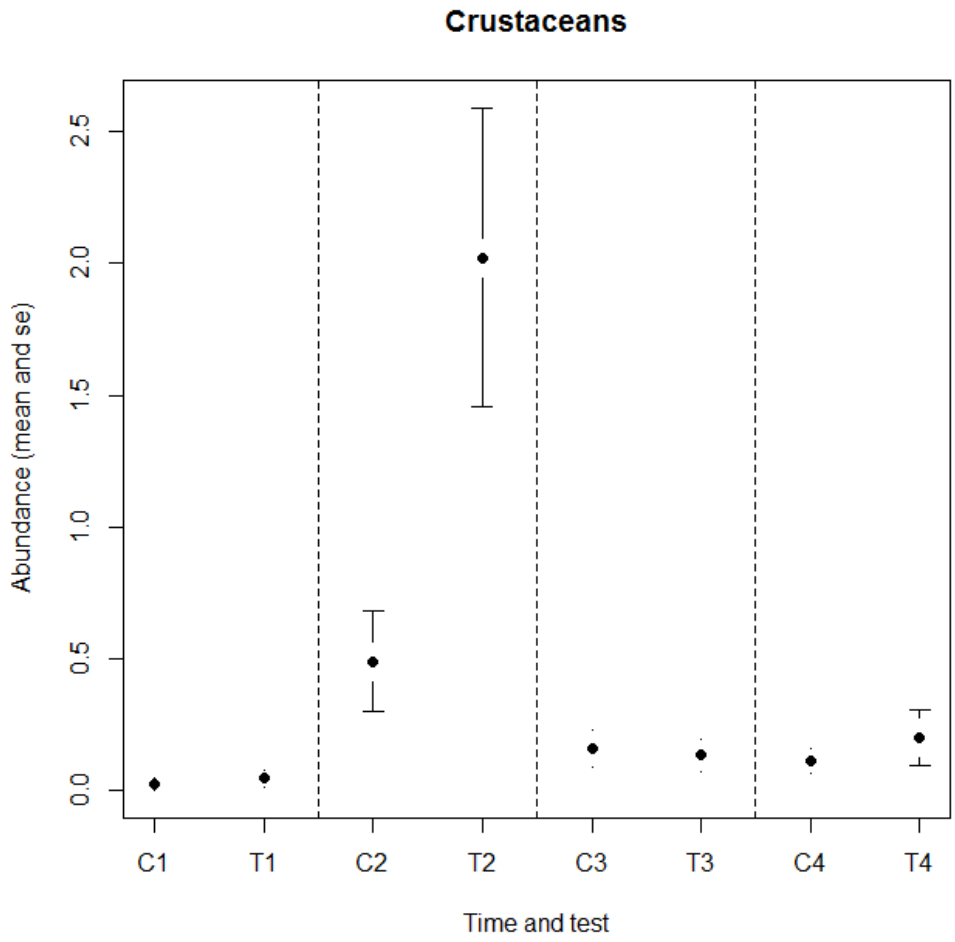


Figure 6.5: Mean abundance of Crustaceans between experimental treatments (C = controls and T = treatment) and sampling times (1 – 4)

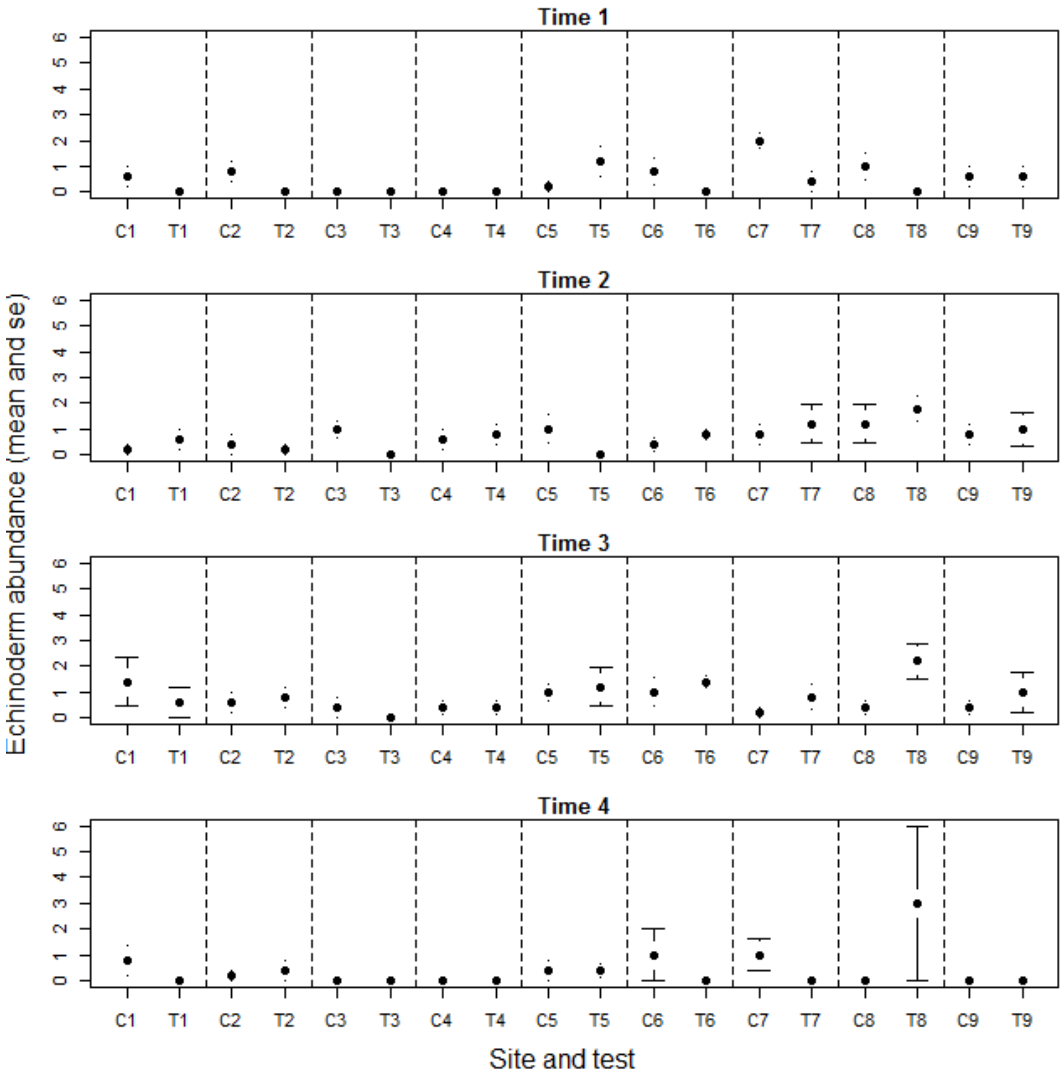


Figure 6.6: Mean abundance of Echinoderms between experimental treatments (C = controls and T = treatment) and sites (1 – 9) for each sampling time (1 – 4)

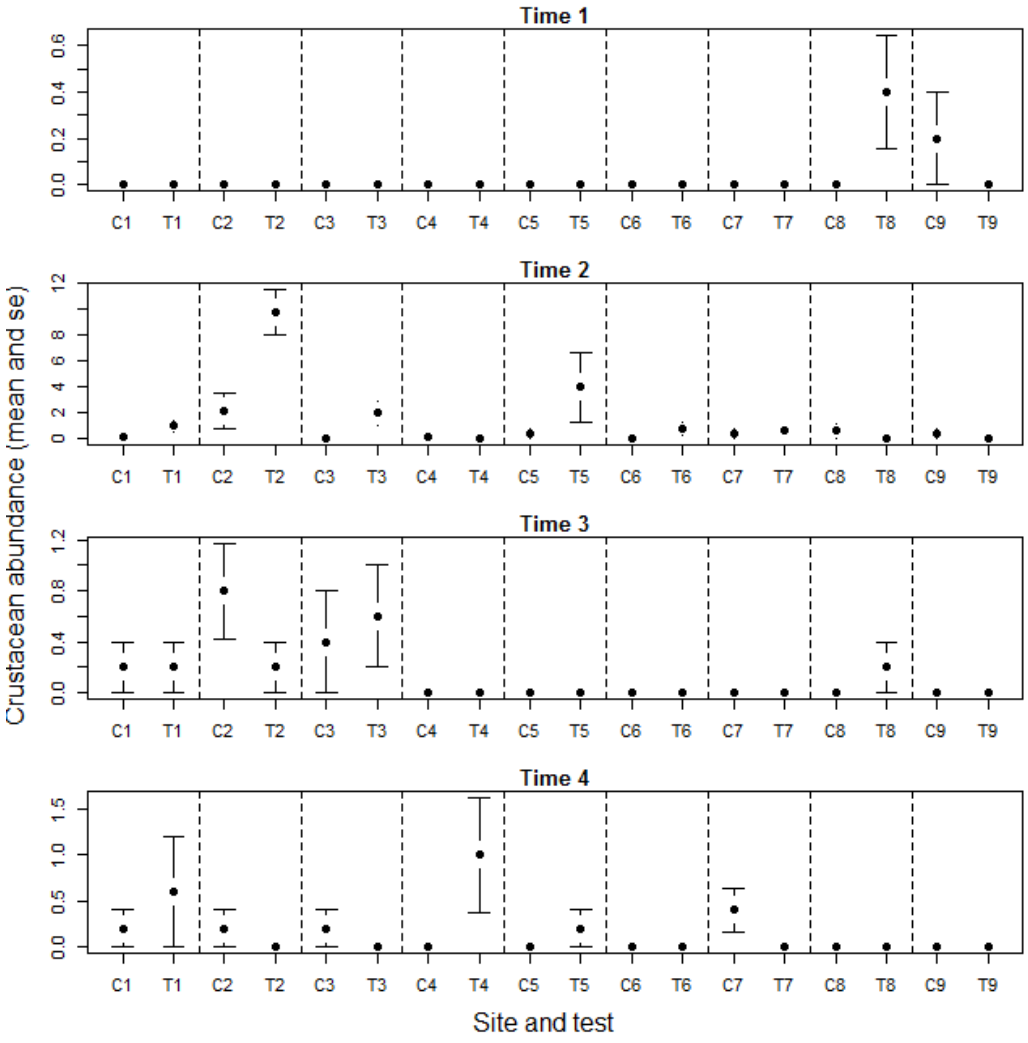


Figure 6.7: Mean abundance of Crustaceans between experimental treatments (C = controls and T = treatment) and sites (1 – 9) for each sampling time (1 – 4)

Discussion

This is the first attempt to quantify the effect of stingray foraging using a manipulative experiment in a tropical marine ecosystem and demonstrated some taxa may be important components in the prey preferences of rays. No taxa were sampled in significantly different abundances between treatment and controls; however, some changes were detected when interactions between factors were examined. Molluscs were sampled in significantly higher abundances in treatments when compared to controls from time 4 suggesting their importance as a prey item for epibenthic predators. All taxa sampled showed significant differences between factor 'time', which may be reflective of natural changes in abundance considering this experiment was conducted over a 12 month period.

Despite the importance of rays as agents of disturbance in benthic environments (O'Shea *et al.* 2012), the results of this exclusion experiment did not conform to my prediction that abundances of infaunal taxa would be higher in ray exclusion zones. Testing indicated non-significant differences between treatments and controls for the main five taxa found. There were however significant two-way and three-way interactions that demonstrated high variation in infaunal abundance within sampling times and sites. There were non-significant interactions for test x site suggesting similar effects were being observed across the study site. Combined, my results suggest that the experimental design did not sufficiently capture the biological variability of this system and possibly the exclusion design was not effective at excluding rays. While no pits were ever detected inside the treatments, there were pits frequently observed on the fringes of the experimental cages. Due to the

relatively infrequent sampling, it is possible that if rays were able to access these treatments, evidence of such feeing activity, such as pits, would be undetectable. The factors structuring tropical benthic communities remain unclear and rays may not be as strong of a structuring force as has been documented in temperate foraging areas. These treatments were designed so other epibenthic predators could still forage within the treatments leaving any effects on infaunal communities attributable to rays only. This design meant that there was potential for immigration and emigration to and from treatments that may have contributed to the non-significant results. Sub-benthic barriers were introduced to a parallel study running concurrently, but this experiment was destroyed by cyclone Bianca in 2011. Future work should include treatments that exclude all bioturbating organisms, and possibly physical barriers to prevent emigration of infaunal taxa.

Bioturbation by smaller organisms such as crabs, gobies and shrimps has been reported to have a greater effect on infaunal abundance than larger predators (Reise 1977). Callianassid shrimps are known to exert a greater physical effect through excavating and foraging behaviour than rays (Myrick & Flessa 1996; O'Shea *et al.* 2012) and it is plausible that rays were simply not causing a detectable disturbance at the spatial or temporal scales measured here. Cross & Curran (2000) compared infaunal abundances between pits and non-disturbed areas, and while they found significant differences between pits and controls, their sampling was rapid and conducted on newly formed pits at low tide, presumably within hours of formation. In addition, other epibenthic predators were still able to function within these treatments, therefore possibly contributing to any effects between treatments and controls.

Despite this, studies of rays as epibenthic predators without manipulations support the idea that they are significant agents of disturbance due to their feeding behaviour (see Peterson *et al.* 2001). This suggests that in Mangrove Bay, rays should potentially be having a greater effect on infaunal communities than detected here. Current studies by O'Shea *et al.* (2012) and Cerutti-Pereya (*unpub. data*) suggest that Mangrove Bay is an important area for juvenile rays and may act as a nursery, while fewer adult (large) rays are detected (O'Shea *et al.* 2012). This potential skew in population structure favouring juvenile rays may account for non-detectable effects because they were either small enough to still feed in the treatments, or juvenile rays are not capable of such disturbance, possibly due to prey handling ability or jaw morphology (Marshall *et al.* 2008).

Few data derived from such experimental manipulations are available, but one such study that examined rays as predators on infaunal communities using experimental manipulation was by Glen vanBlaricom (1982). This study showed that recolonisation of ray pits was largely driven by the accumulation of detritus and other organic matter which acted as foci for amphipods and ostracods on temporal scales of ≤ 24 hours. However, the recolonisation of experimental pits to control levels by the most abundantly sampled taxa took 4 – 6 weeks. If treatments here were effective at preventing rays from feeding, then sampling times may have been too infrequent resulting in a recovery before further samples were taken. While VanBlaricom (1982) reported such rapid re-colonisation, his exclusion cages were all subtidal therefore effectively doubling the soak time and subsequent exposure to epibenthic predators, compared to the exclusions used here, which were in the intertidal zone and thus underwater for only around 6 hours a day.

Another such study, recently published by Ajeman and Powers, (2012) looked at manipulating densities of cownose rays (*Rhinoptera bonasus*) in the northern Gulf of Mexico. These experiments provided a valuable addition to experimental literature, yet results provided no definitive evidence on the effect of ray densities on certain prey populations, most notably amphipod densities. It was concluded that the vast range and highly motile nature of this species confounded results, as experiments were spatially limited. Furthermore, results presented here from Ningaloo Reef may suggest a similar effect, whereby benthic communities sampled at this particular location are not actually impacted by ray predation, as temperate habitats are.

In conclusion, this work does at least provide a preliminary insight that identifies a number of issues that should be addressed in a more comprehensive experimental framework for future research. Such studies should include total exclusion treatments with a greater frequency of sampling, testing of different designs and barriers to emigration. Caging experiments are intended to investigate effects of predation (Posey 1994) by excluding or including potential predators, herbivores or competitors (Connell 1997) and they are ideally suited to small spatial and temporal scales. This removes any confounding environmental effects often found at larger spatial scales (Langlois 2005). These factors should be considered in any future work attempting to quantify ecological effects of predators through experimental manipulation.

Chapter 7:

New Locality Record for the Parasitic Leech *Pterobdella amara*, and two new Host Stingrays at Ningaloo Reef, Western Australia

Introduction

The fish leech, *Pterobdella amara* Kaburaki 1921 is a common parasite previously recorded in *Pastinachus atrus* (cowtail stingray) and *Himantura uarnak* (reticulated whip ray) from India and the east coast of Australia, and *Dasyatis akajei* (Japanese red ray) in Japan (Burreson 2006). *Pastinachus atrus* is an extremely common species of benthic ray recorded from the Indo-pacific and can attain sizes of 200 cm disc width (Last & Stevens 2009). Known as the cowtail stingray on account of a skin fold on the tail, it is not considered harmful to humans and typically flees when encountered making it difficult to observe. This species spends a lot of time concealed below the sediments and can swim off at great acceleration when disturbed and for this reason close and accurate observation can be difficult.

During the course of research at Ningaloo Reef (23°07’48. 28’S, 113°45’44. 70E), individuals of a small population of *Pastinachus atrus* were caught in hand nets in a shallow lagoon and humanely euthanized (see chapter 2). One individual of this species was located in less than 1 m of water, occupying the surf zone without concealment early in the morning at high tide. It made no attempt at evasion and initially it was thought to be deceased because it had lost its dark bronze colouration and instead was pale blue. Confirmation of the species had to be made from the deep skin fold on its tail.

The ray was caught in a hand net where it showed the first signs of movement, which appeared to be slow, and at no time did it display any behaviour to suggest it was attempting to get away. The ray was immediately euthanized and subsequently weighed, measured and dissected. The animal weighed 5 kg and had a disc width (W_D) of 99 cm and a total length (L_T) of 149 cm. Three other comparable individuals of this species taken at this location had W_D of 99 cm, 81 cm and 100 cm (TL 189 cm, 130 cm and 184 cm) and weighed 16, 12 and 21 kg respectively. Upon dissection of the animal, the buccal cavity, gills, inter-nasal flap, pharynx and oesophagus contained high densities (approximately 60 – 100) of gnathiid isopod larvae all of a similar size measuring approximately 4 – 6 mm each (Figure 7.1).

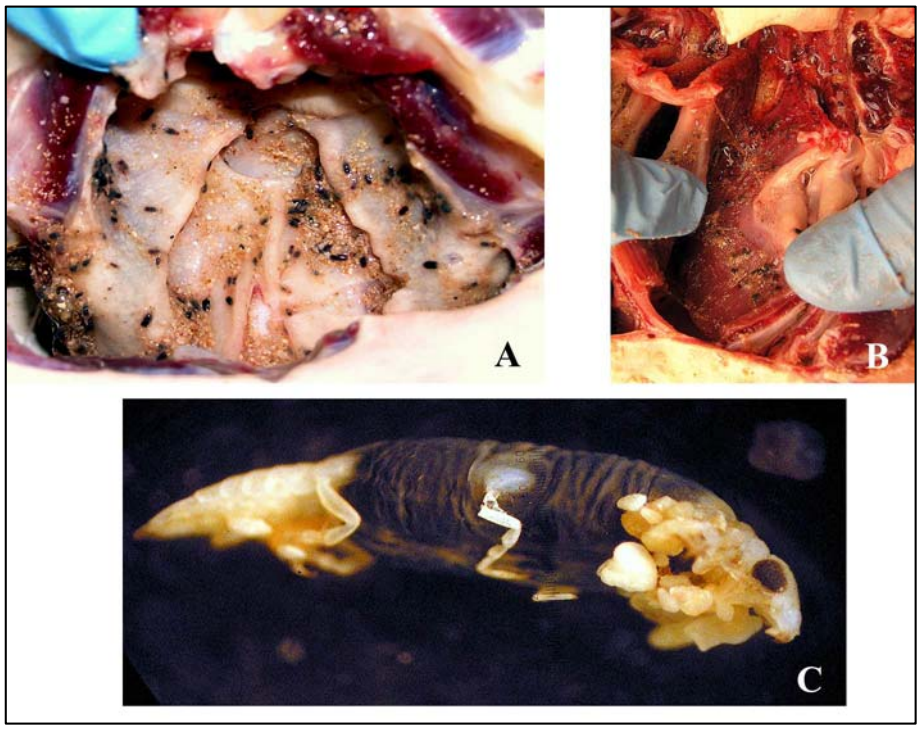


Figure 7.1: (A) Buccal cavity of *P. atrus* with gnathiid isopod larvae embedded in tissue, **(B)** gills of the same individual ray with similar infestation and **(C)** magnified close up of gnathiid isopod larvae

On the jaw of the ray were around 30 individuals of the parasitic leech *Pterobdella amara,* seemingly burrowing outward from an area of basal attachment approximately 1 – 2cm in length (Figure 7.2).

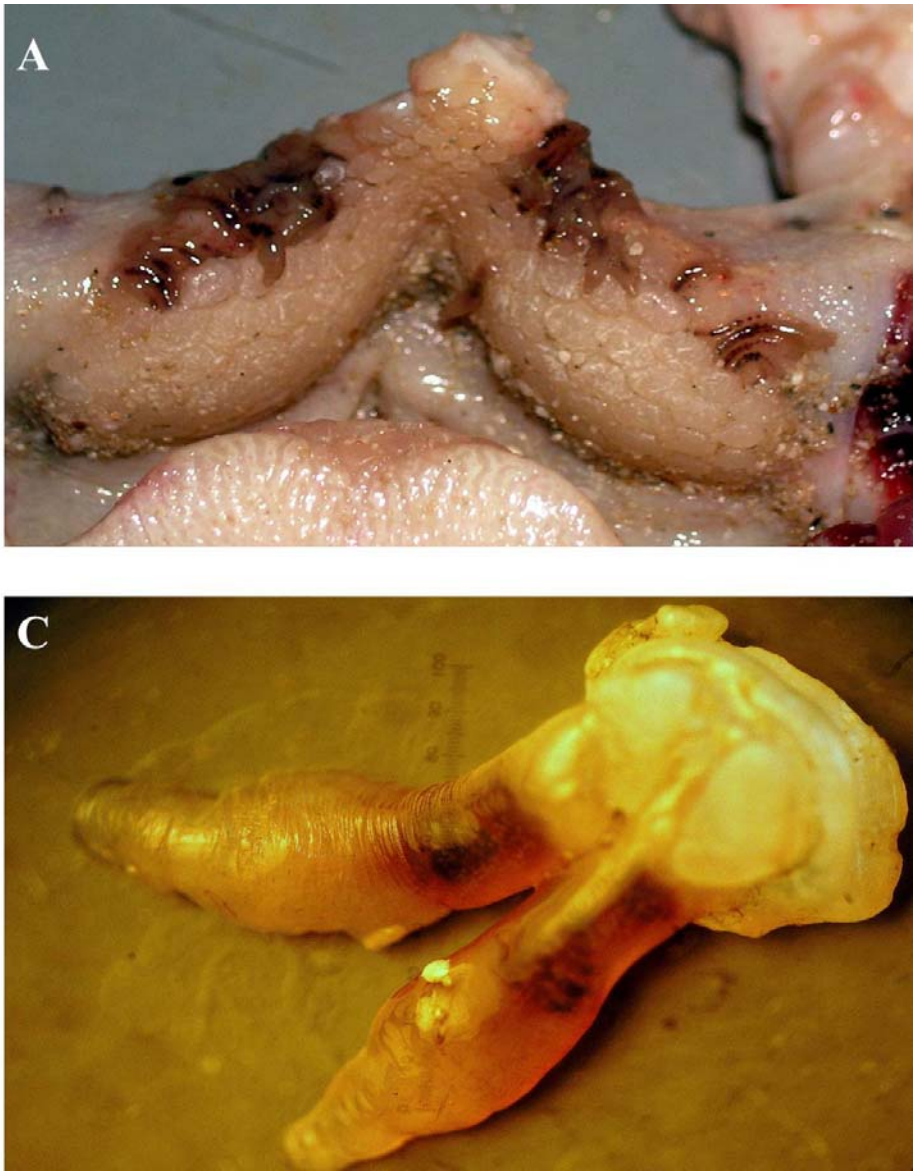


Figure 7.2: (A) Upper dentition of the ray *Pastinachus atrus* showing the parasitic leech *Pterobdella amara* and (**C)** close up of two individual leeches showing the area of basal attachment with part of the stingray jaw still attached

The jaw was in various stages of degradation both at the site of attachment and unaffected areas. Leeches were attached firmly and when removed, areas of jaw used for attachment as well as surrounding parts of the dentition would break away.

Discussion

It is likely that the degradation seen in the jaw was caused by the presence of the parasite. In addition to this, the stomach was empty of any prey item or sediment and was bloated when compared to other comparable specimens. The body cavity also contained a brown fluid surrounding the organs which flowed freely when the initial incision was made, which is common to all further specimens of ray which have been found to host this species of leech (n = 9).

It is hypothesised that the external appearance of this cowtail stingray, the apparent degradation of the jaw and the unusually low body weight was caused by a combination of the leech species and the gnathiid isopods. In addition, this is the first time that this species of leech has been described from Western Australia and has subsequently been lodged in the WA Museum, catalogue number WAM V7613. Previous to this find, very few parasites had been noted on the six species of stingray sampled at this location (n = 170). However, two further species of stingray, the leopard whip ray (*Himantura leoparda*) and the porcupine ray (*Urogymnus asperrimus*) have been found with *P. amara* attached to the jaw, which is a new host record for Western Australia. The parasite specimens from these two new hosts have also been lodged at the WA Museum under catalogue numbers WAM V7709 & 771.

Chapter 8:

Transmitter attachment and release methods for short-term shark and stingray tracking on coral reefs

Introduction

Since the inception of studies using acoustic telemetry to track elasmobranchs in the 1960s, there have been two commonly reoccurring problems faced by researchers: firstly, transmitter retention for the duration of the study and secondly, recovery of the transmitter upon completion. Issues of transmitter attachment are particularly critical for studies that involve active tracking of animals. Unlike passive monitoring using fixed receivers, which may be ongoing for several years (e.g. Heupel & Simpfendorfer 2005; Papastamatiou *et al*. 2009; Speed *et al*. 2011), active tracking invariably takes place over short durations of a few hours to a few days. The aim of such studies is to provide detailed, fine-scale (m) tracks of animals by following the signal from the transmitter (e.g. McKibben & Nelson 1986; Nakano *et al.* 2003; Johnson *et al.* 2009). The intense nature of this field work means that it can usually only be sustained for a relatively brief time (usually no more than a few days) and for this reason, it is vital that a quick method of transmitter attachment is used that minimises stress and does not induce unusual behaviour by the study animal during tracking.

Ideally, active tracking should be long enough to capture the cycle of behaviour of interest; often one or more 24-hour periods in most instances (Nelson1990). In some studies, researchers have trialled feeding transmitters to sharks concealed in

bait, although short retention times and regurgitation of transmitters have complicated this approach (McKibben & Nelson 1986; Economakis & Lobel 1998; Papastamatiou *et al*. 2009). In addition, this method is often not selective in that it doesn't allow the researcher to be selective about size or gender (but see Brunnschweiler 2009), and may not allow the researcher to gain other vital information about the individual being tracked. For these reasons, attaching the transmitter using a barbed dart inserted under the skin of the animal or inserting the transmitter into the body cavity of captured animals by surgery are most commonly used for this work. In particular, external tagging of rays has proved to be problematic on account of their dorso-ventral compression and lack of prominent dorsal fins to which transmitters can be attached (Le Port *et al.* 2008). Using the internal implantation approach also has the problem that it involves considerable trauma for the animals (Nelson 1990). Furthermore, transmitters cannot be retrieved after the tracks are completed, unless researchers are willing to euthanize the subject animals.

A pioneering study by Bass & Rascovich (1965) suggested that a buoyant transmitter attached externally with water-soluble glue or an electromagnet might enhance the likelihood of transmitter retrieval. Since then, a range of technology has been developed for the timed release of external transmitters including electronic burn wires, as well as various types of corrodible links that are activated in seawater. Electronic burn wires enable the user to determine exactly when the transmitter should be released from the animal, although they are costly and for this reason, cheaper alternatives such as galvanic timed releases (GTR) have been used. Galvanic timed releases were initially designed for use in the fishing industry, although they

are now also used by researchers to slowly corrode in seawater and eventually release transmitters attached to animals. However, release times of GTRs can be affected by environmental factors, with warmer water increasing the rate of corrosion and making detachment of transmitters somewhat unpredictable in the tropics. Nevertheless, such technology has been used extensively for tracking sharks in a variety of environments such as: Pacific angel *Squatina californica* (Ayres 1859) (Standora & Nelson 1977), grey nurse *Carcharias taurus* (Rafinesque 1810) (Bruce *et al.* 2005; Bansemer & Bennett 2009), sandbar *Carcharhinus plumbeus* (Nardo 1827) (Rechisky & Wetherbee 2003), white *Carcharodon carcharias* (L. 1758) (Bruce *et al.* 2006), grey reef *Carcharhinus amblyrhynchos* (Bleeker 1856) (Skomal *et al.* 2008) and whale *Rhincodon typus* (Smith 1828) (Gleiss *et al.* 2009) sharks. Tracking studies of marine reptiles such as crocodilians (Franklin 2009) and turtles (Hazel 2009) have also employed the use of GTRs.

The present study focuses on the attachment of external transmitters to reef sharks (Carcharhinidae) and stingrays (Dasyatidae) on a tropical coral reef. We used an external tagging technique because tracking was intended to commence within a few hours of attachment and required that transmitters were retained for up to 24 hours. Furthermore, this method was judged this to be less traumatic for animals than internal tagging, as surgery and restraint of animals for long periods (> 10 minutes) were not necessary. Knowledge of restricted home ranges and repeated daily movement patterns (e.g. Cartamil *et al*. 2003; Collins *et al*. 2007), coupled with the use of external transmitters, also allowed the possibility of transmitter retrieval to be explored using buoyancy aids for the transmitter.

Specifically, the aims of this study were to test whether: 1) external attachment would ensure retention for the duration of the tracking period, 2) using GTRs in a tropical environment would result in reduced retention time compared to the temperate water conditions in which they were designed for, and 3) an attachment design that includes both a floatation device and GTR would permit retrieval of transmitters upon completion of the track and leave minimal equipment attached to animals.

Materials and Methods

Study location

Field tests of GTRs and animal tracking were conducted in the lagoon of Ningaloo Reef, Western Australia near the township of Coral Bay (-23.1335, 113.7703) (Figure 8.1). The lagoon is a shallow (1 – 10 m water depth) habitat, characterised by extensive sand flats, consolidated limestone platforms, and interspersed with coral reef patches. This area is known to have a high diversity of reef shark and stingray species (Stevens *et al*. 2009) and the complex coastline provides sheltered bays, ideal for monitoring short-term movement behaviour of these animals due to protection from the prevailing winds and reduced wave action.

Galvanic timed release static field test

Two B5 GTRs (International Fishing Devices Inc.) were tested in Skeleton Bay, a known shark aggregation site within the lagoon of Coral Bay (Speed *et al.* 2011) between the 9th and 11th of November 2008. This model of GTR was designed and

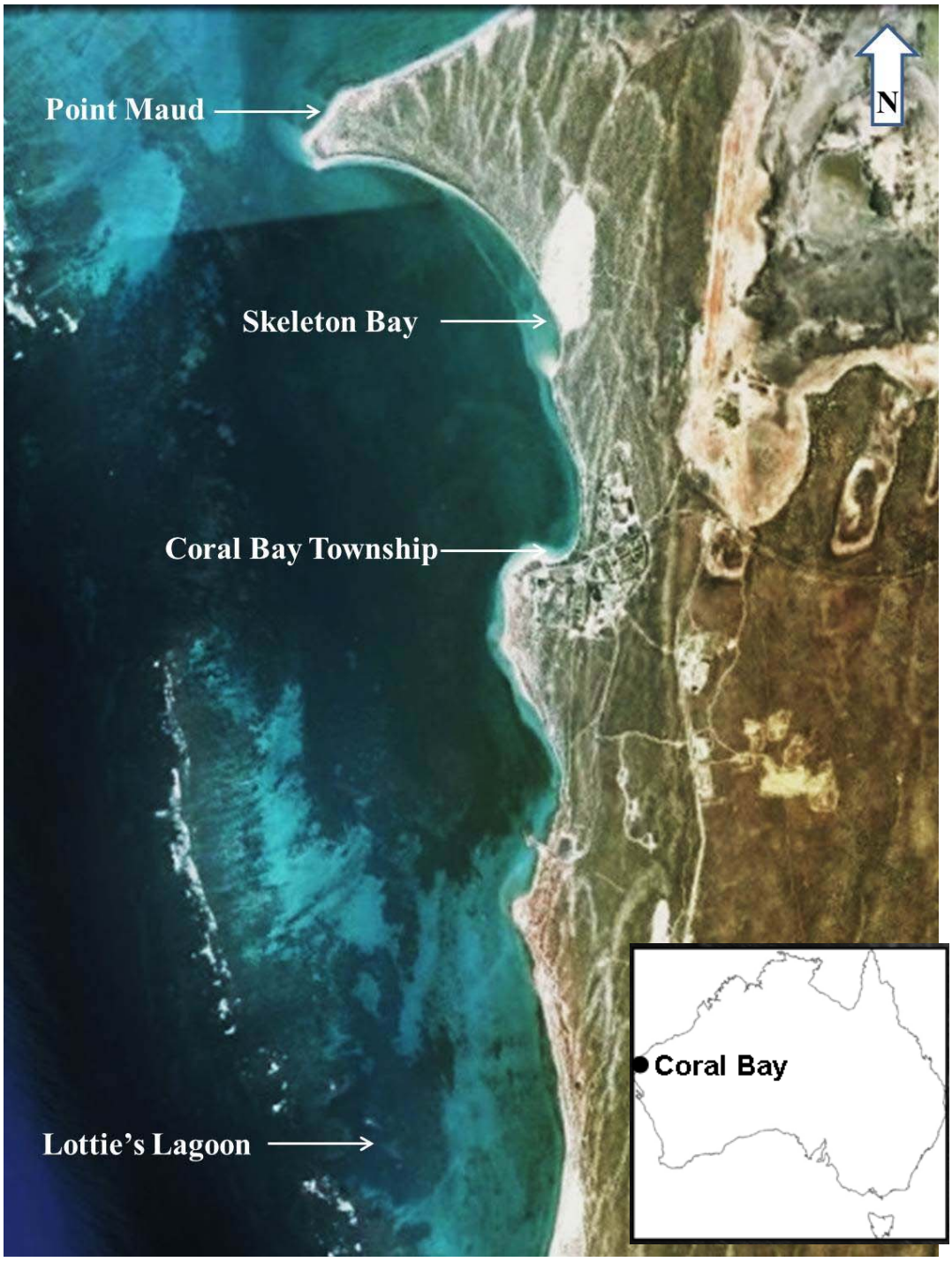


Figure 8.1: Map Coral Bay Township and surrounding reef where tags were tested

tested for water temperatures between 14 – 21°C, and was predicted to corrode after 48 hours according to this model's specifications. Both GTRs were placed on the sand 60 cm apart from one another and checked after 24 hours, 48 hours and 53.5 hours. Each GTR was anchored using a 2 kg lead weight and suspended with 70 kg fishing line, which was attached to an identical float that was intended for use in animal tracking. A plastic zip tie was used to attach the GTR to the weight and an additional anchor line was attached from the weight to the float to avoid losing it once the GTR had released. Water temperature was monitored half-hourly with a temperature logger (VEMCO Minilog) for the duration of the experiment.

Transmitter attachment design and deployment

Reef sharks

Both v16 and v13 continuous acoustic transmitters (VEMCO) were trialled for external attachment on blacktip reef sharks (*Carcharhinus melanopterus*) (Quoy & Gaimard 1824). The end of each transmitter was scored using a P50, medium grit sandpaper and marine putty (Selley's Knead It Aqua) applied to one end. A small hole was formed in the putty and reinforced with steel eyelets. Alternatively, transmitter mounts with built-in eyelets are also available from VEMCO.

Transmitters were then connected to a B5 GTR with 70 kg monofilament nylon fishing line (~ 20 cm long) and conical fishing floats. The line was also bound at either end of the exposed portion of the transmitter with two small cable ties, to avoid the transmitter resting and rubbing on the animal during tracking. The opposite end of the GTR was fastened to the female part of a Jumbo Rototag by a cable tie,

which threaded through a hole that had been drilled in the tag (Figure 8.2). The tag was then applied to the first dorsal fin as per standard application of Jumbo Rototags© (Kohler & Turner 2001).

Prior to tracking, the ranges of continuous acoustic transmitters were tested in Skeleton Bay during August 2009. Both the v13 and v16 models were tested with a VR100 receiver and VH110 (VEMCO) directional hydrophone and it was determined that the maximum range was between ~ 280 and 300 m (see details in Speed *et al.* 2009). Sharks were caught at the beach adjacent to Skeleton Bay using ~ 115 kg monofilament handlines with baited barbless hooks (see details in Speed *et al.* 2011). Acoustic transmitters were then attached to the first dorsal fin using the Rototag applicator, after having first taken a dermal punch to avoid splitting the fin.

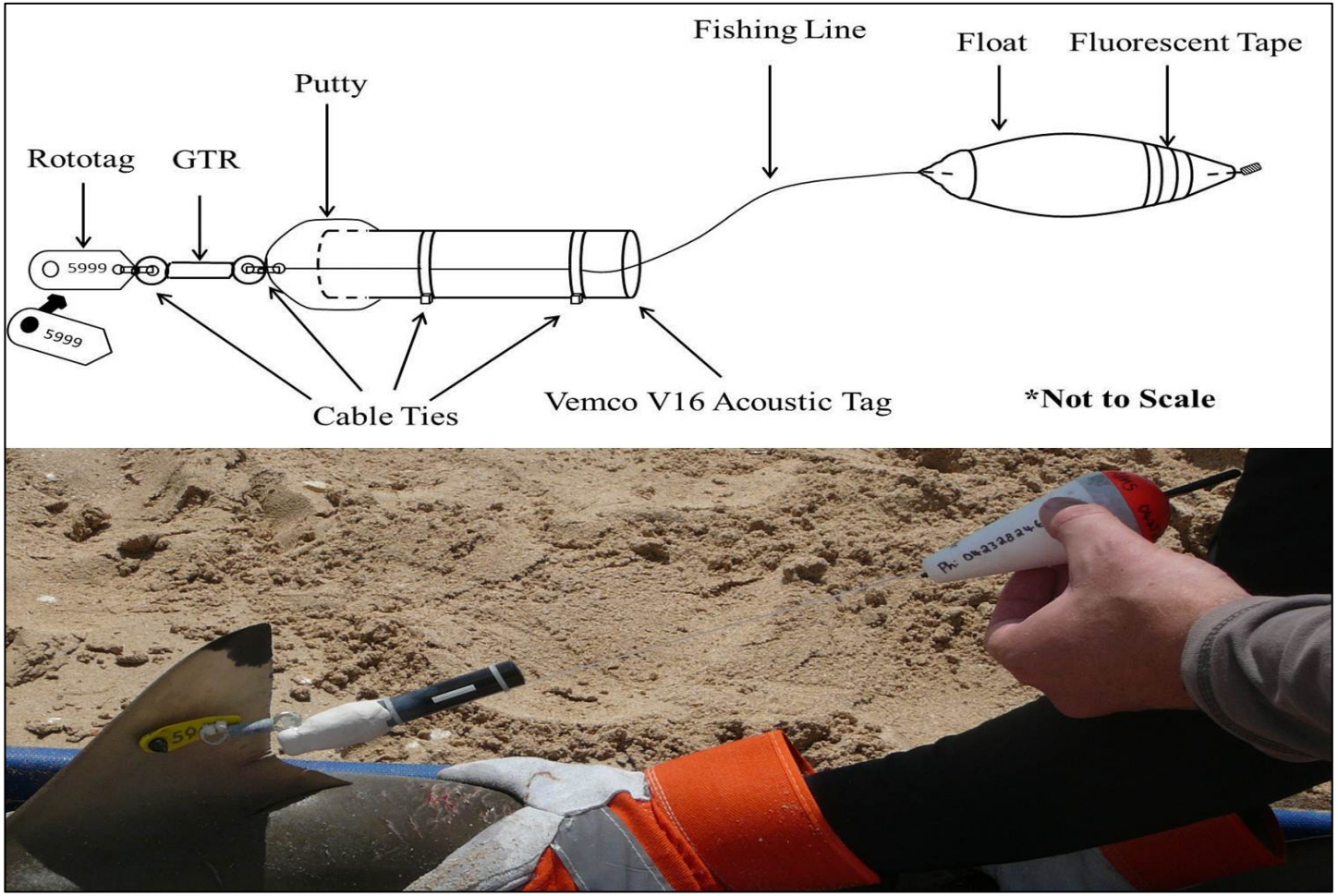


Figure 8.2: External attachment design for active tracking of reef sharks

Stingrays

Transmitter preparation and rig design for stingrays differed slightly to the methods used for sharks, and was designed for use on both cowtail rays *Pastinachus atrus* (Forsskål 1775) and porcupine rays *Urogymnus asperrimus* (Bloch & Schneider 1801). Putty was attached to the float as well as the GTR, which was directly embedded within the putty and allowed to set overnight. A wire trace was looped directly through the GTR and crimped in three places before being surrounded in a thermal plastic sheath. This length of wire trace would be left after the transmitter would release, so it was designed to be as short as possible in order to have the least impact on the animal post-release. The other end of the wire was looped once more through a stainless steel dart and held in place by one of the crimps (Figure 8.3).

Large dasyatid rays (W_D > 100 cm) were tagged using VEMCO v16 continuous transmitters attached externally using a Mares Cyrano 700 Pneumatic Spear gun that was modified to mount transmitters. The head pin (manufactured by Exmouth Light Engineering, Exmouth, Western Australia) allowed the mounting of the transmitter by securing the dart in a groove and trailing the wire tether and transmitter along the shaft, which was attached by a rubber band to prevent it floating off when in the water. Transmitters were attached to rays in their pectoral fins close to the central disc. To avoid penetrating the entire fin and causing potential harm to the animal, a cork was placed at the base of the pin shaft so that it could only penetrate to a fixed depth. This distance could be adjusted to suit the size of the ray. Animals were tagged from directly above while snorkelling and the transmitter was fired around 30 – 50 cm from the ray.

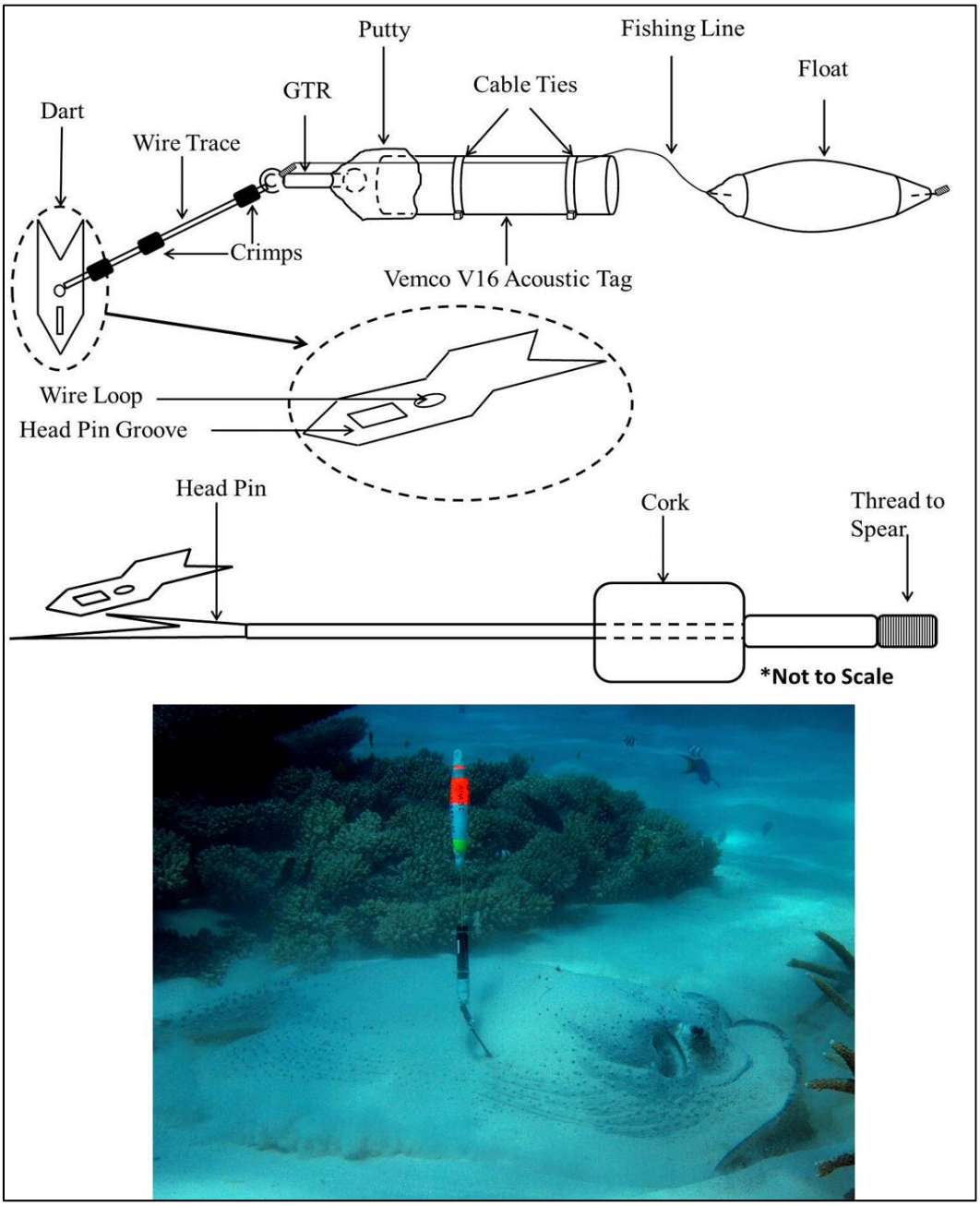


Figure 8.3: External attachment design for active tracking of stingrays. *Photo shows porcupine ray Urogymnus asperrimus tagged at Lottie's Lagoon.*

Shark and stingray tracking

Sharks and rays were tracked using a VR100 (VEMCO) ultrasonic receiver and a VH110 (VEMCO) solid aluminium directional hydrophone. The hydrophone was attached to the end of a telescopic pole that was fixed to the gunnel of a 4.5 m monohull boat with an outboard engine, similar to the method described by Holland & colleagues (1992). A distance of ~ 10 m was maintained to animals where possible to ensure strong signal reception from the transmitter and also minimise observer influence on animal behaviour. Visual confirmation of shark presence during the day was possible due to clear, shallow waters and was also possible at night due to reflective tape on the float. Rays were tagged in less than 6 m of water in sandy, lagoonal habitat within the Maud Sanctuary Zone, east of Lottie's lagoon and were all feeding when tagged.

Tracking began before 10 am on each of three days and lasted for up to 9 hours, while one of these rays was also tracked a second time after re-location of the animal 24 hours after the initial track. No night tracks were possible due to bad weather. If transmitter signals were lost during tracking, a VH165 (VEMCO) omnidirectional hydrophone was used to provide a broader scan of the surrounding area in order to re-establish the position of the animal so that tracking could be resumed using the directional hydrophone. Signals received from the transmitters were recorded in decibels (Db) every 3 seconds, which corresponded to an approximate distance of 0 (no detection) to 100 (full detection and very close proximity). Coral reefs can create echoes or 'phantom' signals due to topographic complexity, although typically a signal of 90 – 100 Db corresponded to approximately 0 – 15 m distance from transmitter to hydrophone, which was also validated during field-testing.

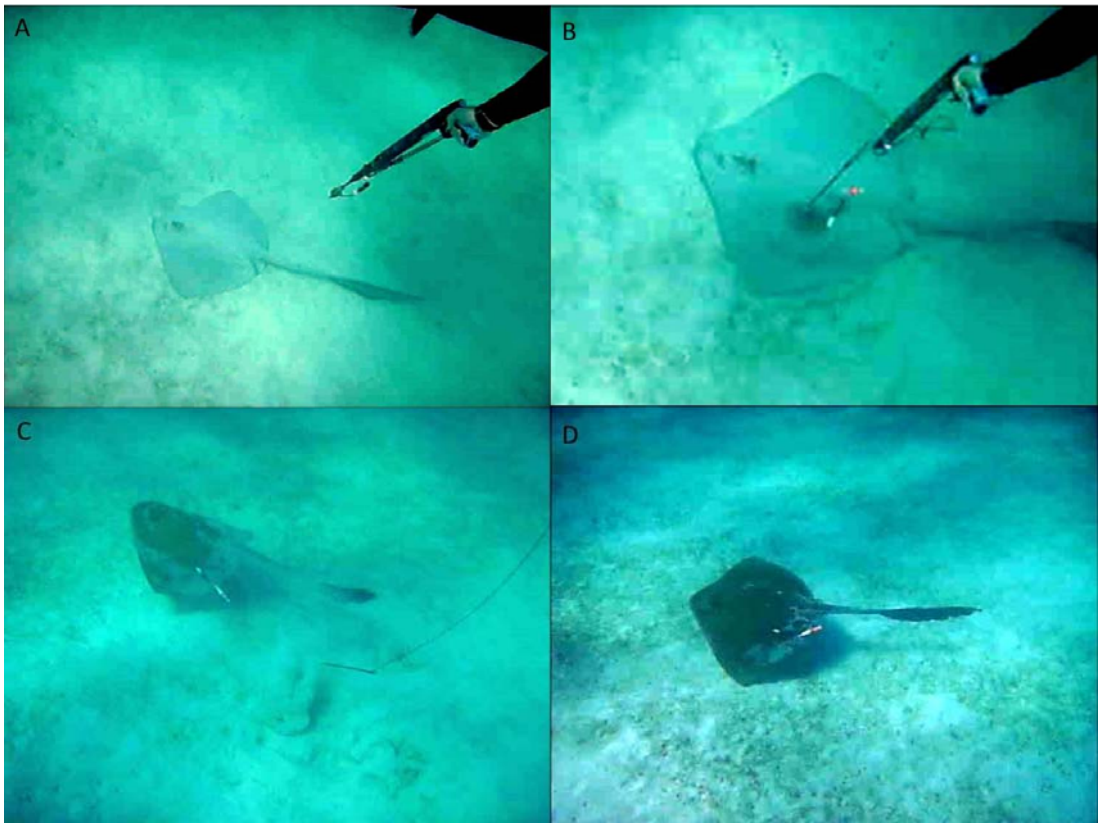


Figure 8.4: Optimal technique for in-situ tagging of large benthic rays with (**A)** approach from the side and slightly from behind; (**B)** Tag attachments from directly above in the pectoral fin close to the body; (**C)** The movement of the ray swimming away provides the detachment of the tag from the spear and (**D)** the ray swimming away with the tag attached correctly with minimum invasion

Two simple methods were used to plot the tracks of sharks and rays. The first method, used for plotting shark position data, used only positions that had > 90 dB recordings, which corresponded to animals being in close proximity to the boat. The average of these positions was taken for each hour and plotted in ArcMap. The second method, used for plotting stingray tracks, involved standardising all position data by dividing each track into 30-minute intervals (600 waypoints per 30 minutes)

and then one waypoint for each half an hour block of each track was randomly chosen using a random number generator to be plotted in ArcMap.

Transmitter retrieval

In order to facilitate transmitter retrieval, our contact details were put on the float of the rig to enable the public to return a transmitter if it was found after it had detached from an animal and washed up onto a beach. In addition, we searched beaches both north and south of where each animal was tagged in an attempt to locate transmitters after the 48 hour attachment period had elapsed.

Results

Galvanic timed release static field test

Both GTRs were still attached after 24 hours, which was the minimum time required for shark and ray tracking purposes. One of the GTRs had prematurely released by 48 hours and the other released between 48 and 54 hours (Figure 8.5). The minimum, mean and maximum water temperatures recorded for the duration of the experiment were 20.8C°, 23.3C° (± 0.97 SD) and 24.7C° respectively. The mean water temperature throughout the experiment was therefore warmer than the water temperature range for which this model of GTR was originally designed (14 – 21° C).

Transmitter attachment design and deployment

The procedure of transmitter attachment for sharks took approximately five minutes from capture to release. All transmitters remained attached to animals for the duration of tracks and neither design appeared to inhibit movement. Once released, only the Rototag with a single nickel ring from the GTR remained attached to the

animal. The use of reflective tape on floats provided an effective means of confirming the location of tagged sharks at night using a flashlight from the boat. Each stingray was tagged successfully on the first attempt, which took no longer than 20 seconds. Once the transmitter released, the stainless steel dart and a wire trace (~ 6 – 10 cm in length) were left on the animal.

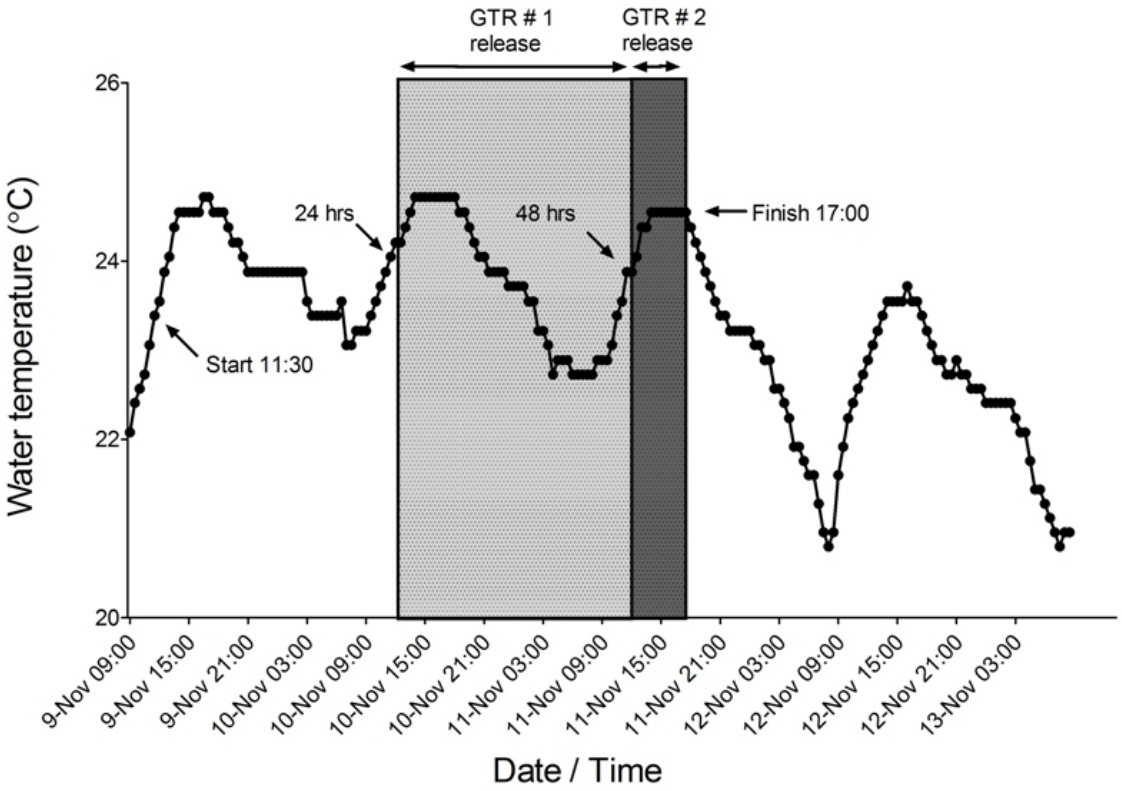


Figure 8.5: Water temperature and GTR release times for experiment duration

Stingray tracking

Three adult rays were successfully tracked, the first of which was a female cowtail ray of approximately 100 cm W_D that was tagged at 09:57 and tracked for 8 hours and 47 mins (Figure 8.6A and Table 8.1). The same ray was tracked again when the signal was re-established 24 hours later and followed for a further 7 hours and 12 mins. The second animal was also a female cowtail ray that was tagged at 08:30 and

tracked for 8 hours and 33 mins. The last track was obtained from a porcupine ray tagged at 10:00 and tracked for 8 hours and 50 mins. While each ray remained relatively sedentary during the tracking periods, some small-scale movements (10 – 100 m) were made, with the greatest activity occurring around dusk. Immediately after tagging, the ray was followed by snorkeler for as long as possible with the boat following at a distance. This was to ensure the welfare of the animal post-tagging and also to confirm correct transmitter placement and attachments. After tagging, each ray settled on sand immediately adjacent to coral complex within 30 – 50 m of the tagging site. One porcupine ray remained stationary for almost 8 hours in the same location, before moving at dusk. During these stationary phases, observations were made by a snorkeler every 60 minutes to confirm the transmitter was still attached. In each case, feeding was observed suggesting 'normal' behaviours had resumed post-tagging.

Shark tracking

Two adult female blacktip reef sharks were successfully tracked, which were both tagged at the beach adjacent to Skeleton Bay. The first track lasted > 20 continuous hours, commencing at 14:48 pm and finishing at 11:26 am the following morning. The second track covered a two day period and was split into two separate tracking periods of 4 hours 44 mins on the first day and 6 hours 28 mins on the second day (Table 8.1). Both animals remained within the lagoon during the tracking period and the first largely moved within Skeleton Bay (Figure 8.6B). It was not possible to plot the track for the second shark owing to an insufficient number of clear detections. However, continuous signal detections were maintained throughout the respective tracking periods, although animals became difficult to track at low tide due to

patches of exposed reef. Tracks were terminated due to either inclement weather or signal loss at low tide.

Table 8.1: List of species tracked with acoustic telemetry. **Shark size is presented in total length (L_T) and ray size is presented in approximate disc width (W_D)*

Species	Size* (cm)	Sex	1st track	2nd track
C. melanopterus	161	F	20 hr. 22 mins	NA
C. melanopterus	133	F	4 hr. 44 mins	6 hr. 28 mins
P. atrus	100	F	8 hr. 47 mins	7 hr. 12 mins
P. atrus	100	F	8 hr. 33 mins	NA
U. asperrimus	110	F	8 hr. 50 mins	NA

Transmitter retrieval

A single shark transmitter was retrieved from a beach by a member of the public near the deployment site in Skeleton Bay. The other transmitter was not relocated after the completion of the subsequent shark-tracking period. All three transmitters deployed on stingrays were retrieved by members of the public walking along the beach of Bill's Bay approximately 4 – 5 km north of the tagging locations.

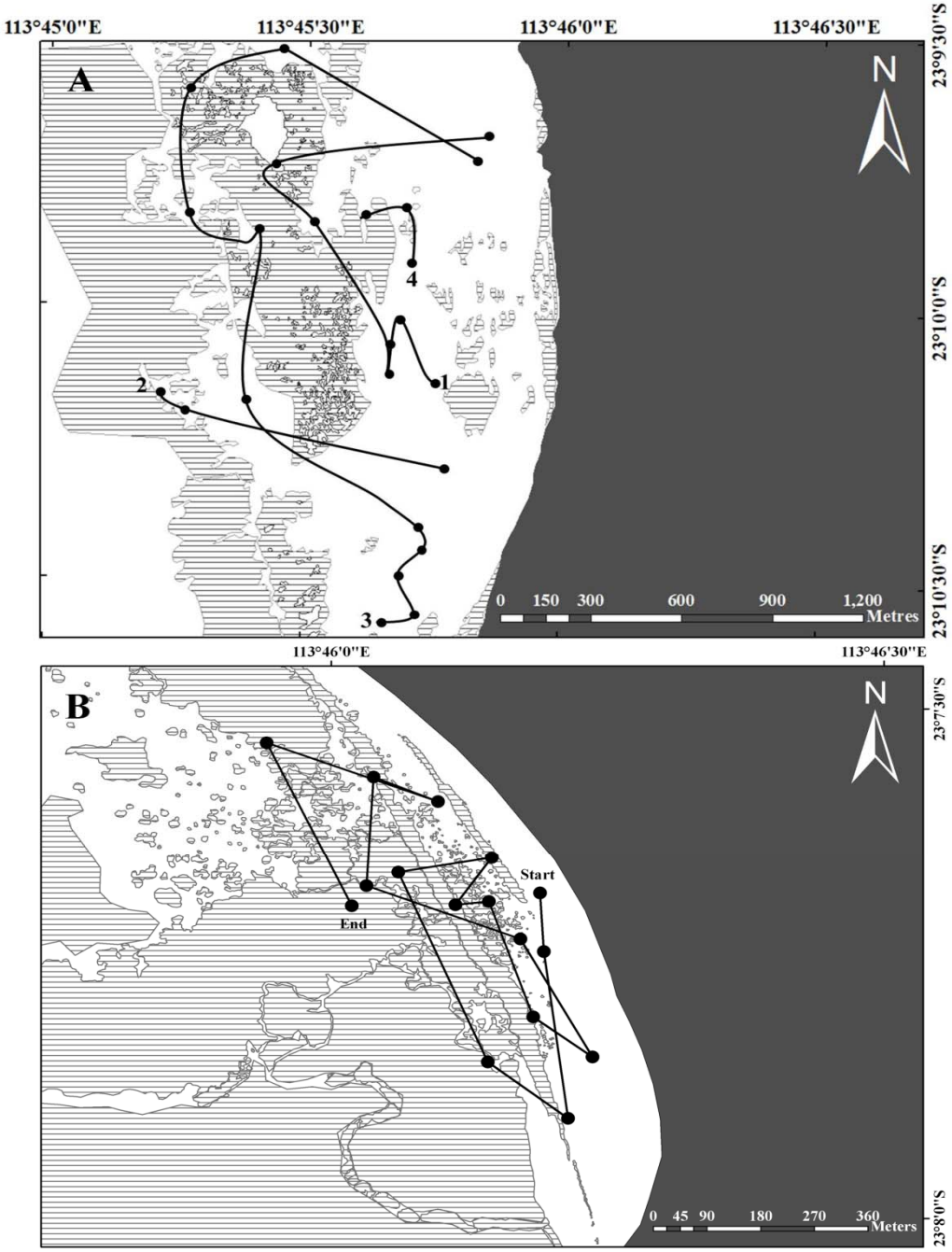


Figure 8.6: (A) Stingray tracks up to nine hours duration each, within the Maud Sanctuary Zone, Ningaloo Reef; numbers represent the starting points of each track and the points represent locations at 30 minute intervals, and **(B)** Track of *C. melanopterus* within Skeleton Bay, Ningaloo Reef; each point represents an average position for each hour tracked

Discussion

Although the technical difficulties associated with tagging and tracking elasmobranchs in tropical reef environments are well recognised (e.g. Nelson 1977, Bres (1993), Simpfendorfer & Heupel 2004), the solutions to these problems are not always reported in detail within the literature. This can result in each new study having to re-invent through trial and error new solutions to the same problems. The current study is an attempt to build on existing concepts of transmitter attachment and act as a guide for researchers that are unfamiliar with such techniques. Moreover, given the proliferation of acoustic telemetry studies of elasmobranchs over recent decades (e.g. Nelson 1990, Sundström *et al.* 2001), it is incumbent on researchers to find and disseminate the most effective and least intrusive methods of tagging and tracking. A simple, cost-effective external attachment with a galvanic timed-release for transmitters allowed the successful tracking of sharks and stingrays in our study with minimum disturbance to animals, and in most instances retrieval of transmitters.

All transmitters remained attached to animals for the predicted deployment period however, detachment times were more variable. Even in static trials when GTRs were exposed to identical environmental factors, detachment times varied by several hours. For the current study, this was not an issue but might be an important consideration in situations where the retrieval of transmitters requires greater release time accuracy. Overall, release times remained broadly consistent with the predicted duration of 48 hours stated by the GTR manufacturer for water temperatures between 14 and 21° C, suggesting that the use of GTRs for studies in tropical waters remains valid.

The use of modified Rototags on blacktip reef sharks provided a rapid and moderately invasive attachment technique for transmitters. The permanent placement of the Rototag served for identification of previously tagged animals once acoustic transmitters had detached. Early telemetry studies of reef sharks suggested that external attachment of transmitters might have caused abnormal behaviour due to trauma (Nelson & Johnson 1980), although recent work on juvenile bull *Carcharhinus leucas* (Müller & Henle 1839) and lemon sharks *Negaprion brevirostris* (Poey 1868) advocated external attachment because it was relatively quick and less invasive (Yeiser *et al.* 2008) than surgical implantation of transmitters within the animal. A recent tracking study used internal tagging for blacktip reef sharks, although the researchers waited for at least 48 hours prior to tracking to avoid changes in behaviour due to tagging trauma (Papastamatiou *et al.* 2010). The concurrent use of our external tagging technique might provide a means by which the extent and duration of 'abnormal' behaviour due to the trauma of internal application of transmitters might be empirically tested. Regardless of the tagging method adopted, it is generally advocated that data collected from immediate post-tagging is removed from analyses in behavioural studies, which is generally the first few hours of tracking (Review by Sundström *et al.* 2001).

Early studies on blue sharks *Prionace glauca* (L. 1758) found that on release, tagged individuals immediately dived below the thermocline, which was thought to be a response to the trauma of external attachment of transmitters (Sciarrotta & Nelson 1977; Carey & Scharold 1990). Similarly, a recent study that trialled a new technique of external attachment of motion sensors to whale sharks observed comparable post-tagging behaviour (Gleiss *et al.* 2009). In the current study, tagged

blacktip reef sharks made rapid movements away from the tagging location upon release, although appeared to resume 'normal' behaviour within an hour. In the shallows of Skeleton Bay, this consisted of slow swimming in water depths of 1 – 2 m over the sand, which has been documented in a previous study using visual censuses (Speed *et al.* 2011). Likewise, stingrays swam away from the snorkeler immediately after tagging, but resumed behaviour that was consistent with feeding within a few minutes. Nonetheless, future studies to confirm these behavioural observations are required.

Nelson (1990) suggested that there was a trade-off between the immediate effects of the trauma of internal implantation of transmitters and the long-term irritation of an external transmitter rubbing against the skin. Long-term effects of irritation were not an issue in our study due to the brevity of tracking, although longer tracking periods (multiple weeks) with the same design would be possible with longer-lasting models of GTRs. For both stingrays and sharks, transmitter attachment design in the current study meant that there was only a small likelihood of abrasion on the skin. A further benefit of this design is that little instrumentation remains on animals after transmitter detachment, which could potentially attract bio fouling and lead to infection (e.g. Jewell *et al*. 2011), although internal implantation techniques would remove the potential for bio-fouling. Earlier work has shown that the Rototags do not have long-term negative effects on the health of blacktip reef sharks (Heupel *et al.* 1998).

A number of transmitter attachment methods for stingrays have been trialled without negative effects to animal welfare including: inserting monofilament line through the

tail to attach PSAT tags (Le Port *et al.* 2008), cinch tags attached to the spiracular cartilage (Collins *et al.* 2007) and a braided nylon harness passed through the spiracles in order to tow a transmitter (Blaylock 1990). In all cases, these attachment techniques require capture and restraint of animals, with the risks inherent for the researcher in handling potentially dangerous species as well as the trauma to the animals. In-water tagging using a spear gun as described here is not without risk, but arguably such risks, both for the researcher and the animal, are considerably reduced when there is no requirement for capture and restraint (Review by Sundström *et al.* 2001). Spear guns and spears have been used to deploy external transmitters on large elasmobranchs in situations where restraint of the animal is not possible due to practical or ethical issues, for example on nurse sharks *Ginglymostoma cirratum* (Bonnaterre 1788) (Pratt & Carrier 2001), manta rays *Manta birostris* (Donndorff 1798) (Dewar *et al.* 2008) and whale sharks (Wilson *et al.* 2006).

The opportunistic retrieval of transmitters once they had detached was partially due to the limited spatial scale (10 s – 100 s of m) of movements and site fidelity of subject animals, which is common in many reef associated species of elasmobranchs (e.g. Chapman *et al.* 2005, Lowe *et al.* 2006, Wetherbee *et al.* 2007, Dewar *et al.* 2008, O'Shea *et al.* 2010, Speed *et al.* 2011), as well as favourable wind and wave conditions. VHF radio transmitters have also been used previously to locate devices once they had detached from animals when at sea (e.g. Gleiss *et al.* 2009, Houghton *et al.* 2009). Future models of our rig could include a VHF transmitter within the float, which would increase the likelihood of tag retrieval at sea or once washed up on a beach.

Methods of attachment of acoustic transmitters in the current study provide both alternative and complementary techniques to internal implantation and/or ingestion of transmitters by animals. However, the goal of future tagging studies should be to find an attachment method for transmitters that is completely non-invasive and involves as little risk as possible both for the researcher and the subject animal. Hopefully, such innovation will be developed and current advances in transmitter attachment will continue to be disseminated within the research community.

Chapter 9:

General Discussion

Dasyatids of Ningaloo Reef

Dasyatid rays were encountered in high abundances within the Ningaloo Reef Marine Park, indicating the importance of this habitat for this group of fishes, and supporting previous suggestions that elasmobranch populations here may be the largest and most diverse found anywhere on the Australian coastline (Stevens *et al.* 2009). This study represents the first assessment of the ecology and biology of batoid fishes and will serve as a foundation for further studies on elasmobranchs at Ningaloo.

Although we are yet to obtain a complete picture of the ecological role of stingrays (Heithaus 2004), they are known to fulfil several functions. These include modifying the physical habitat in soft sediment environments though bioturbation; potentially exercising a top-down control within the biological community structure of infaunal invertebrates via predation; and further providing linkages within the food web as mesopredators. To date most of this information comes from temperate habitats, while the role of rays in tropical marine ecosystems remains largely unstudied. This lack of information is critical because in these latter habitats, small-scale and artisanal fisheries in developing countries often target rays.

Understanding basic biological parameters and processes forms the cornerstone of research for a given species, particularly if they are considered commercially valuable. These data provide crucial foundations for stock assessments, which are required to mitigate susceptibility to fishing pressure; however, many ray species

remain data deficient (Smart *et al.* 2012). Commonly, if a species is of little commercial value, samples are harder to acquire and this acts as a barrier to research. In Australia, rays are not valued commercially, which has led to a general deficiency in biological data for many species. This is exacerbated by certain species being elusive or cryptobenthic and therefore harder to detect in conventional fisheries-independent surveys (Pierce & Bennett 2009). Bycatch reduction devices have proven effective at excluding large rays from catches since their mandatory use in the early 2000s (Brewer *et al.* 2006), which has resulted in rays becoming harder to procure for research purposes. This thesis has attempted to remedy many of these issues by documenting ecological functions, biological parameters and life history characteristics of numerically abundant, yet data deficient stingrays at Ningaloo Reef.

The main intention of this thesis was to provide baseline data for stingrays within a coral reef environment and to highlight their significance as an influential species. Species studied here are found in the broader Indo-Pacific bioregion where they are often targeted by artisanal fisheries and it is hoped that this research can provide the foundation for management and conservation efforts for these and similar species.

Main findings

Lethal sampling of stingrays for research

Due to the complex nature of the various habitats within Ningaloo Reef, and its protected status as a marine park and World Heritage area, conventional methods of stingray collection such as commercial trawling are not possible. In order to attain the required sample sizes to successfully describe the ecology and biology of rays at Ningaloo, spearguns were used to catch and kill large stingrays. This research

complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC 1990) and was granted approval by the Murdoch University Animal Ethics Committee. An obligation to obtaining this approval was to prepare and present a methods summary detailing the humane destruction of stingrays as part of this PhD research. This chapter detailed the reasons for developing such a program; methods used to ensure safety for researchers and humane destruction of stingrays; and also discussed pain perception in elasmobranchs. Many ray species are difficult to sample, particularly those of no or little commercial value, which cannot be obtained from fisheries. Stingrays at Ningaloo are one such group and through the methods detailed in this chapter it is hoped that any future study that faces similar challenges can draw from these techniques to ensure safe working practices and ethical treatment of stingrays, or any other large, benthic elasmobranchs. The overall outcome of this study was that almost all of the rays caught were believed to have suffered minimally based on the skilful and rapid methods in which they were lethally sampled; there is also evidence suggesting that pain perception is likely to not occur in elasmobranchs in the same way it does in higher vertebrates (Sneddon 2009).

Optimising age and growth parameter estimates in poorly represented ray species

Four of the ray species studied (*Himantura uarnak*, *Neotrygon kuhlii*, *Pastinachus atrus* and *Taeniura lymma*) could be aged reliably. Realistic interpretations of growth parameter estimates were possible because a multi-analytical approach was adopted, including – for the first time in batoids – a Bayesian framework to increase the precision of estimates generated from a maximum likelihood model. Comparisons of conventional growth models using a likelihood framework indicated

that the two-parameter von Bertalanffy model best described parameter estimates for all species. This result is consistent with published descriptions fitting age and growth curves for small sample sizes (Cailliet & Goldman 2004, Thorson & Simpfendorfer 2009). The Bayesian model included running 1 million iterations or 'steps' to create posterior distributions that refined precision estimates of best fit generated from the model, confirming its viability for small sample sizes. Overcoming small sample sizes in biological studies is imperative in ensuring results are robust enough to have accurate meaning (Heupel & Simpfendorfer 2010). This is particularly important for rare, elusive or endangered species and methods described here can be used in such instances, where conventional assessments are not viable.

It was found from *k* value comparisons that the two larger-bodied species grew more slowly and attained greater ages than the two smaller-bodied species. Both these results are consistent with previously published estimates (Jacobsen & Bennett 2011, Yigin & Ismen 2012); however it has been suggested that species with lower *k* values (< 0.1) are more vulnerable to extrinsic pressure, such as over fishing (Bransetter 1990). The two larger (and slower growing) species – *Himantura uarnak* and *Pastinachus atrus* – both had *k* values above this threshold (0.12 and 0.16 respectively). The growth rates of the two smaller species were estimated at 0.38 (*N. kuhlii*) and 0.24 (*T. lymma*); this was comparable with estimations for *N. kuhlii* from southeast Queensland (Pierce & Bennett 2009), where $k = 0.13$ and 0.20 for females and males respectively.

Despite these parameter estimates for *k* being higher than the threshold suggested by Bransetter (1990), they are still slower growth rates when compared to estimates for other elasmobranchs; for example, the milk shark *Rhizoprionodon acutus* ($k = 0.64$)

and spadenose shark *Scoliodon laticaudus* (k = 1.0) (Kasim 1991); the Australian sharpnose shark *Rhizoprionodon taylori* (k =1.3) (Simpfendorfer 1993); Atlantic sharpnose shark *Rhizoprionodon terraenovae* (k = 0.8) (Carlson & Baremore 2003) and the southern stingray *Dasyatis americana* (k = 0.5) (Henningsen 2002). Despite conservative life history traits, this study suggests that rays sampled from Ningaloo are slow growing, even compared to other elasmobranchs. Furthermore, k value estimates were above the published threshold estimate, suggesting these rays may not be as vulnerable as other species to impacts and fishing pressures; however, this study suggests that these species may still be vulnerable in areas where fishing pressure exists.

This chapter provides valuable information on age and growth characteristics of three previously unreported species of stingray and offers and insight into the age structures of rays within Ningaloo Marine Park. Additionally, the importance of seeking alternative methods for estimating growth parameters cannot be understated, particularly for elasmobranchs that are consistently reported as facing major declines in population abundance.

Dietary characteristics of stingrays at Ningaloo Reef

Few studies have characterised the diets of tropical dasyatids, yet these data are valuable when assessing the mechanics of dietary partitioning and overlap. As part of this study and a parallel PhD research project (Cerrutti-Pereya 2012), several key areas within Ningaloo Reef have been identified as locations where rays aggregate to feed. Through the methods outlined in Chapter 6, it was possible to create an environmental baseline of infaunal taxa at one such site to enable a comparison with the dietary characteristics of five species of ray. Four species of rays (*Neotrygon*

kuhlii, *Pastinachus atrus*, *Taeniura lymma* and *Urogymnus asperrimus*) had a high degree of overlap largely determined by their equal consumption of annelids. While *Himantura uarnak* also consumed annelids, they were far less abundant than other prey species and instead, crustaceans were present in significantly higher amounts than all other prey recovered from stomach samples, suggesting *H. uarnak* as a specialist feeder. This was determined by comparing abundances of crustaceans with the infauna samples from Chapter 6, which supports the disparity in abundance as an indication for feeding specialisation by *H. uarnak*. Discrepancy in dietary content between *H. uarnak* and the four other species does indicate a degree of dietary partitioning considering these species are sympatric, but the scale of partitioning needs to be qualified.

Previous assessments of the feeding ecology of elasmobranchs demonstrate that partitioning of resources is common and allows co-existence of similar species (McEachran *et al.* 1976, Platell *et al.* 1998*a,* Wetherbee & Cortés 2004, White *et al.* 2004, Marshall *et al.* 2008). At Ningaloo, while species were co-occurring in the same location, it is likely some division of resources is occurring along specific ecological axes. Commonly food and then space are partitioned first (White *et al.* 2004); however, availability of certain prey types may be limited by other factors, such as diel/nocturnal emergence, which may correlate with patterns of predator activity. This seems probable for *H. uarnak*, where individuals were never observed feeding during the day. While dietary specialisation within individuals among a guild of predators is generally considered a mechanism for reducing competition (Vaudo & Heithaus 2011), here it may be as a result of an increase in crustacean abundance at night when it seems likely *H. uarnak* is actively feeding.

Evidence suggesting dietary overlap in the four remaining species is indicative that competition is low, which may be explained by rays partitioning resources on a scale other than a trophic one. Of these four species, two are smaller bodied (*Neotrygon kuhlii* and *Taeniura lymma*), attaining maximum sizes of 47 and 35 cm W_D respectively (Last & Stevens 2009), and are more reef associated than the two larger species (O'Shea *et al. unpub. data*). This could explain why these rays are able to share common prey items; while smaller species were feeding among the reef structure, larger species were observed feeding away from the reef. Furthermore, larger species by their nature have the capacity to move among habitats on a greater spatial scale than smaller bodied species, allowing access to prey in a broader spatial context. So, while results here suggest a lack of competition among these four species, it may be that competition is not an issue because resources are partitioned across spatial scales. Furthermore, it may suggest that prey are not limiting and are simply abundant enough to allow competition without dietary partitioning (Vaudo & Heithaus 2011). This study provides the first insight into the dietary characteristics of rays at Ningaloo and suggests which prey taxa are important to these species of ray, in terms of dietary characterisation.

Bioturbation

For the first time, this study has evaluated the impacts on the physical environment related to feeding by demersal rays within Ningaloo Reef Marine Park. Many earlier studies detailing impacts associated with feeding by demersal rays have focused on biological associated changes within benthic communities; for example, Reidenauer & Thistle (1981), VanBlaricom (1982), Merkel (1990), Findlay *et al.* (1990), Hines *et al.* (1997), Thrush *et al.* (1991), Thrush (1999), Cross & Curran (2000). Studies

describing the physical impacts of epibenthic predation have typically focused on species other than stingrays; for example, shore birds (Cadée 1990), callianassid shrimps, (Myrick & Flessa 1996) or alpheid shrimps (Holmer & Heilskov 2008).

Bioturbation is a critical process within marine soft sediment environments due to the importance of benthic habitats providing up to half the nutrients for primary production in coastal seas (Lohrer *et al.* 2004). It has been proposed that broad-scale losses of bioturbating organisms could impair marine ecosystem function (Lohrer *et al.* 2004); it is therefore essential to identify key areas where bioturbating taxa aggregate in large numbers. In this part of my thesis I examined rates of pit formation and persistence and quantified the amount of sediments being excavated by stingrays within Mangrove Bay intertidal sanctuary zone. Rays move into the bay with the flooding tide and observations suggest they spend the high tide foraging. Furthermore, Cerutti-Pereyra (*unpub. data*) suggested juvenile rays use Mangrove Bay as a nursery area where they possibly also seek refuge. This seems likely considering 80% of pits measured were relatively small < 100,000 cm^3 (Supp. Figure 2.1 – Appendix 2).

In addition to predation, rays bioturbate through resting and predation avoidance behaviours, where they excavate sediments to bury themselves. If Mangrove Bay also functions as a nursery area for juvenile rays, it is likely that these two behaviours are contributing to the physical effects reported here. So, given the significance of rays as bioturbators in coastal marine habitats and their abundance within lagoonal habitats such as at Ningaloo Reef, it seems somewhat surprising that this is one of only a few attempts to quantify this process. While ray bioturbation is clearly an intense activity at Mangrove Bay, the area of ray pits accounted for only

2.42% of the sampled area, despite the probability of pit presence being relatively high each day. This is consistent with other published accounts however, which suggest that rays, while contributing to bioturbation rates, are less important than other taxa, such as burrowing shrimps (Myrick & Flessa 1996). It has further been suggested that physical processes such as wave action may be contributing more to the shifting sediments in certain habitats than ray feeding, although this is likely to be site dependent (VanBlaricom 1982). If the rays within Mangrove Bay are mostly juveniles, then it seems plausible that their effect as bioturbators may not be as significant as that of larger rays, or other taxa that are more prolific such as crabs or shrimps.

This study has assessed one of the ecological roles that rays fulfil within the Marine Park, using Mangrove Bay as an example; however, throughout the course of this study, other areas were identified that supported large aggregations of rays. This was anticipated given the complex geomorphology of the coastline and numerous shallow, intertidal embayments, lending further support to the importance of Ningaloo Reef as a habitat for stingrays.

Experimental manipulation of rays

There are numerous anecdotal reports of biological changes brought about by the predation behaviour of rays, yet attempts to experimentally investigate these impacts are rare. Experimental manipulation can be a powerful tool in assessing roles of predators in structuring marine ecosystems (Hall *et al.* 1990) and is a relatively common approach in marine ecological studies. Manipulations can range from short term and small scale, for example caging experiments over a season (VanBlaricom 1982, Matsuda *et al.* 2009), to larger, longer term manipulations, like the creation of

marine reserves for indefinite periods (Dayton *et al.* 1998). Here, for the first time in a tropical ecosystem, ray densities were manipulated to prevent feeding in specific areas using exclusion cages to detect changes in abundance of infaunal taxa.

The central hypothesis of this study was that infaunal abundances would differ significantly between exclusion treatments and non-caged controls. These experiments assessed numerical abundances of infaunal taxa for the first time within the Marine Park and also indicated which species were important for stingrays using this area to feed. Mangrove Bay has been identified as a site of high ray feeding activity (Chapter 5) and as such was deemed a good location to investigate the feeding impacts of rays.

The experimental results from this study propose that rays are having an effect on some, but not all infaunal taxa that were sampled. This is somewhat surprising considering the level of feeding activity reported for this area from Chapter 5, and the results VanBlaricom (1980) reported in terms of ray feeding efficiency; yet may be explained in part by acknowledging that this site is dominated by juvenile rays. Jaw morphology has been used to explain ontogenetic dietary shifts in rays (Marshall *et al.* 2008) and this may have some relevance here, where prey handling ability or foraging depth are restricted by life history phase or body size. Despite this, it is commonly considered that most infauna dwell in the upper 3 – 4 cm of sediment (VanBlaricom 1978) and the results of Chapter 5 suggested that the mean foraging depth by rays was 5.6 cm, which should encompass most infauna. Experimental pilot cores taken prior to commencement of this study indicated that the anoxic layer was approximately10 cm deep on average in this part of Mangrove Bay. This lends further credence to the notion that these rays are likely to be clearing

most of the infauna due to foraging depth, but the overall lack of significance between treatments here may be an artefact of individual ray sizes. Additionally, this lends support to the theory that these smaller rays, while still prolific bioturbators, are only producing a detectable biological effect when operating as part of a larger guild of bioturbating taxa.

New locality records

A somewhat unexpected component of this research was the observation of parasitic leeches within the buccal cavities of three species of ray caught as part of the lethal sampling program. Of the 170 rays euthanized, most had intestinal parasites without any visible detriment to animals' wellbeing; however, nine proved to be hosting a leech not yet recorded in Western Australia. Furthermore, two species of ray found to host this leech were also new distribution records for Ningaloo. Of these nine individuals, eight were seemingly in good health when captured and observations of the leech were made post-mortem. There were no large deviations in weight of these individuals when compared to conspecifics without the leech present. However, one individual was underweight when compared to others of similar size and it was assumed that the presence of these parasites related to its apparent poor health. The symptoms observed in this study were closely mirrored by symptoms reported in the yellow stingray *Urobatis jamaicensis* after deliberate infestation by another species of parasitic leech *Branchellion torpedinis* (Marancik *et al.* 2012). These included being severely underweight for their sizes. These leeches have oral suckers that attach basally to the dentition in fishes, resulting in dental degradation and therefore presumably directly affecting the hosts' ability to feed. It is likely the discolouration

in the individual I caught, the brown fluid in the body cavity and heavily swollen stomach, were a result of malnutrition.

There are many published accounts of parasitism in the marine environment, mainly dealing with cleaning symbioses (O'Shea *et al.* 2010) or using parasites as 'tags' in population studies (MacKenzie 2002). Brooks (1981) used parasites to assess the biogeography of South American stingrays from the known cladograms of helminths, which led to interest in the evolutionary relationship among parasites and their hosts. However, there are very few published accounts of parasite-host relationships in stingrays. Diamant & others (2010) reported a new microsporidian parasite on the common stingray *Dasyatis pastinaca* from the Mediterranean which was the first record of this species described as a parasite of a batoid. Yamauchi & Ota (2012) also reported a new locality record and host relationship for the leech *Stibarobdella macrothela* parasitising the ray *Taeniura meyeni* in Micronesia. New locality records for parasites and host relationships are rare and most appear to be opportunistic discoveries. Despite this, there is scope for further research of these relationships within Ningaloo Marine Park to assess the extent to which these leeches and other parasites are impacting rays at individual and population scales.

Short-term tracking of sharks and stingrays on coral reefs

There has been a recent and rapid increase in published work detailing the spatial ecology and movement patterns of a variety of marine creatures, including tuna (Josse *et al.* 1998), whales (Clark *et al.* 1986), lobsters (van der Meeren 1997) and even box jellyfish (Gordon & Seymour 2009). This is largely due to advances in technology, making tracking devices smaller and more cost effective. It is however elasmobranchs that often receive the greatest attention due to the documented global

decline in shark populations, particularly in coral reef ecosystems (Robbins *et al.* 2006). Tagging and tracking elasmobranchs using acoustic transmitters has long been recognised as a valuable tool in ecological studies (Kohler 1988), yet problems still exist when using this technology; in particular tag retention and recovery. To overcome these problems, this chapter detailed the trialling of simple, cost-effective measures to evaluate whether short-term tracks were viable in a coral reef environment, as well as tag recovery upon the completion of the track. Test results were also included which not only support the methods, but also further served as preliminary results for short-term tracking of sharks and stingrays at Ningaloo. The concept of using galvanic timed releases for this method of tag attachment proved successful in static field tests as well as practical application when tagging sharks and rays. Two separate methods were developed and described for sharks and stingrays; tag retention performed as expected, while tag recovery was somewhat dependent on currents, winds and opportunistic tourists; four of the five tags deployed were recovered. Tagging of sharks was more controlled as the animals were restrained, however, for the rays, tags were fired into the musculature of the pectoral fin of free-swimming animals using a pneumatic speargun, so the scope for tag loss and injury to the animal was greater; however no visible injuries were sustained by the animals.

Overall, the short-term nature of the tracks served to support the methods, but issues arise in monitoring an animal for 24 hours, where its behaviour may be modified by the act of tagging. For the shark species tagged here, ‘normal’ behaviour was observed; i.e. active movement over a relatively small area through the night with observations detailing hunting and feeding. For rays, quantifying behaviour was less

clear; being relatively sedentary animals, most of their tracks were spent buried in sand, and the movements recorded in this study were brief transitions from one point of the reef to another and did not represent continuous movements. It is very likely that some form of trauma is experienced when some species are tagged (Kohler & Turner 2001) and so defining 'abnormal' behaviour post tagging/release, while difficult, needs to be considered. The difference between shark and ray tagging in this manner is that the trauma experienced by sharks is likely not to extend beyond the capture process; the fitted tag penetrates the cartilaginous dorsal fin, whereas rays have tags punctured into musculature with a force that clearly disturbs the rays.

These results provide preliminary indicators of fine spatial scale, i.e. hundreds – thousands of metres, habitat use by the tagged species that can now be developed further. More importantly, this chapter is the first to detail novel, reliable and cost effective methods for achieving tag attachment in sharks and rays for short-term monitoring in a coral reef, i.e. hours-days. Recovery potential of tags in certain environments means there is the possibility of re-deployment of tags, increasing the cost effectiveness for recording fine-scale movement patterns of marine fishes.

Future directions

While this thesis has identified several key processes and functions of stingrays inhabiting Ningaloo Marine Park, it also provides scope for further work. Bioturbation by juvenile rays is clearly important at Mangrove Bay and this study now needs to be replicated among several locations including those where large rays have been identified. This will allow a more complete determination of this process and its related ecological significances across broader spatial and temporal scales. Future work should possibly combine these methodologies with a more robust

experimental assessment of the biological impacts rays are having through predation behaviour. The exclusion experiment suffered from the same limitations as the bioturbation study, in that the area studied was dominated by juvenile rays and therefore may not be truly reflective of the impacts that rays, as a diverse group of epibenthic predators, are having within the these coastal environments.

Despite these constraints, these results clearly provide valuable information for juvenile rays at this location and can be used as a basis for future work addressing these processes. As part of this study, an experiment was set up using sub-benthic barriers to prevent immigration and emigration of infaunal taxa from the treatments and a 'floating lid' designed to exclude other epibenthic predators. Initial observations indicated that rays were also excluded after large pits were located all around the treatments but never in them. While the study was not completed (due to storm damage), it was anticipated that this experiment would have provided viable results for comparison, and this provides justification for future work in experimental assessments of stingray feeding.

Future work addressing the ecology of rays will benefit from different techniques to address the role of rays within the trophodynamics of the environment, rather than on a species level. Supplementing existing work, stable isotope analysis would allow a more comprehensive assessment of the trophic role of stingrays within the lagoonal food web. This would allow other aspects of ray ecology to be assessed, including predator-prey relationships, considering rays are possibly important prey items for larger sharks within Ningaloo Reef. Speed & colleagues (2011) assessed the trophic role of reef sharks in this manner, and so applying this method to rays would allow

better understanding of the trophic relationship between apex and mesopredators at Ningaloo.

The ageing of tropical stingrays has been rarely studied; Chapter 5 has addressed some of the gaps in current knowledge by providing ages and growth parameter estimates of three previously unreported species (*Himantura uarnak, Pastinachus atrus* and *Taeniura lymma*). While sample sizes were below optimum, this chapter also outlines an analytical method that still produced viable growth parameter estimates. These methods can be used in future work for rare or vulnerable species where large sample sizes are very difficult to obtain

Conclusions

This research has identified key ecological processes in which rays impact upon these environments, and further highlights their importance in coastal and nearshore ecosystems at Ningaloo Reef and other tropical ecosystems. For the first time, the physical effects of bioturbation by rays have been quantified, allowing managers to understand the importance of habitats where rays aggregate; at Ningaloo this is typically shallow, protected (from wave action) intertidal embayments. This thesis also describes the first assessment of impacts associated with ray predation behaviours using an experimental manipulation. Despite numerous limitations, it provides a pilot study for future work to experimentally assess the biological impact rays have on benthic communities. This study has provided baseline data on infaunal communities within Mangrove Bay and as this area has previously been identified as an area of ray aggregation and high ecological value. The calculation of a prey specificity measure has demonstrated the importance of certain invertebrate taxa as key prey items for rays at Ningaloo, while providing a baseline for invertebrate

diversity and abundance within Mangrove Bay. This research has also addressed issues associated with estimating biological parameters from small sample sizes and is the first to use a Bayesian framework to obtain reliable growth parameters in batoids. Results are consistent with previously published estimates of ages and growth rates lending credence to these methods.

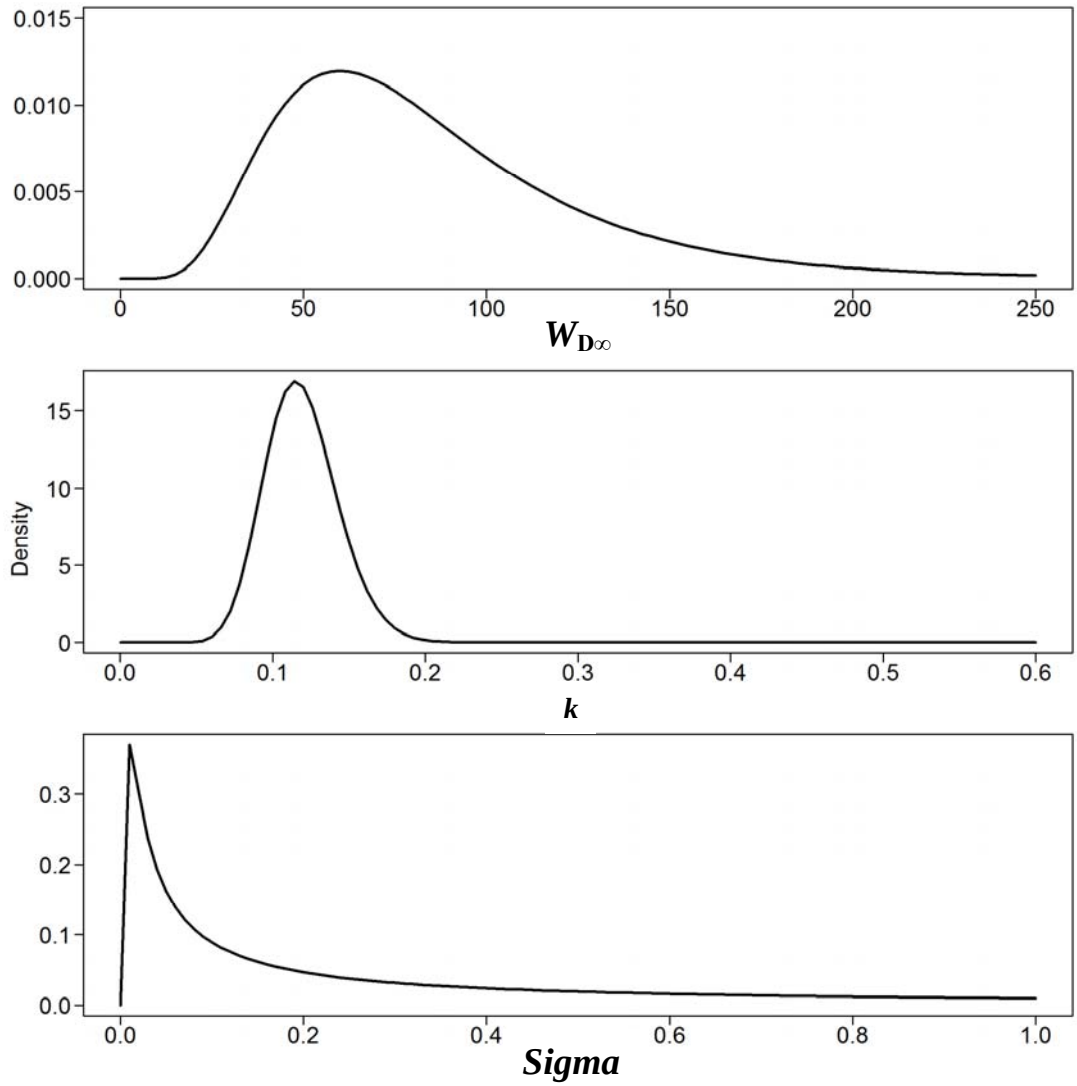
Overall, this study has provided a critical insight into this abundant and ecologically significant group of benthic fishes at Ningaloo Reef. The results presented are relevant in a broader tropical perspective as well as emphasising the biological and ecological value of the Marine Park.

Appendices

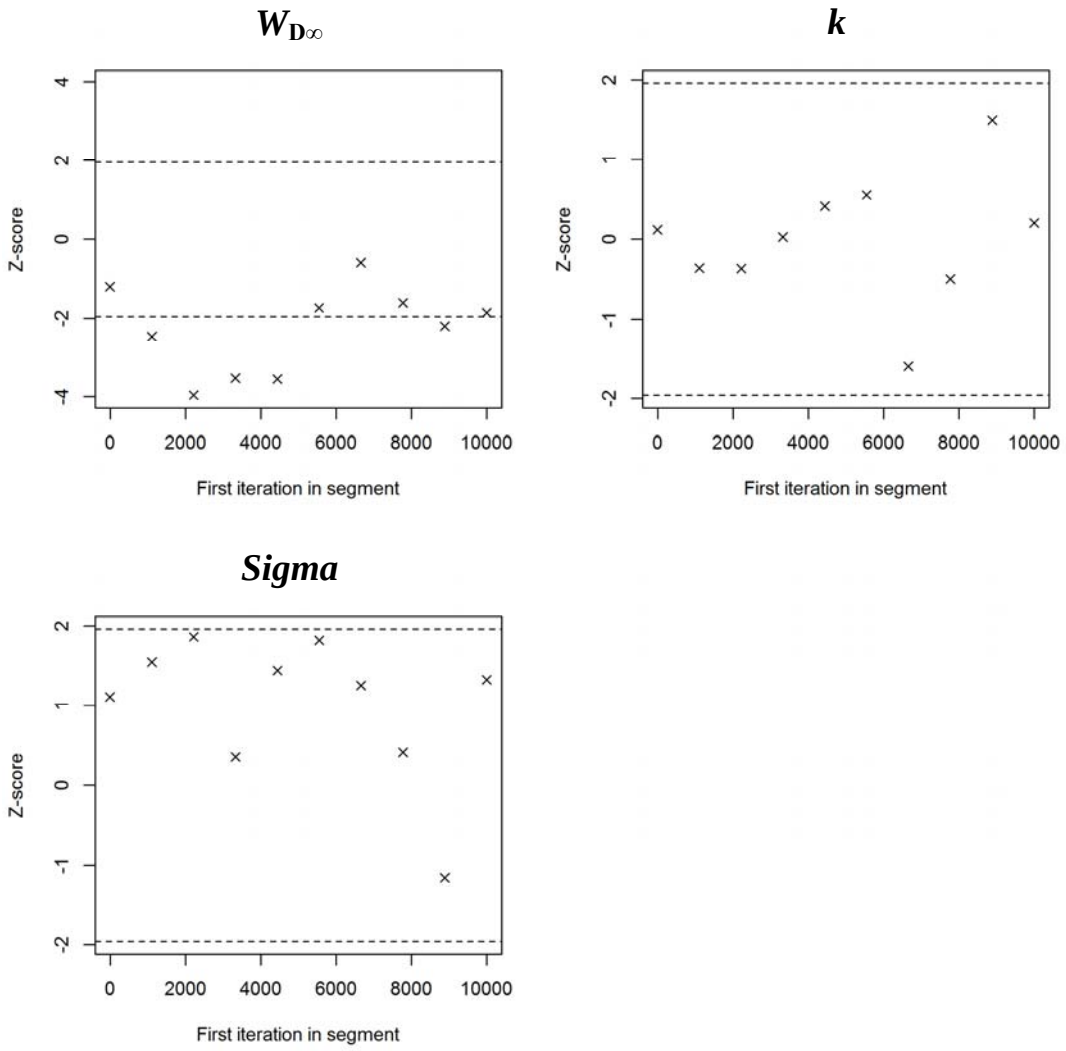
Appendix 1:
Supplementary Tables and Figures to Chapter 3

Supplementary Table 3.1: Species and mean growth parameter estimates of W_{Dmax}, $W_{D\infty}$ and k derived from published sources and used as informative priors in the Bayesian model

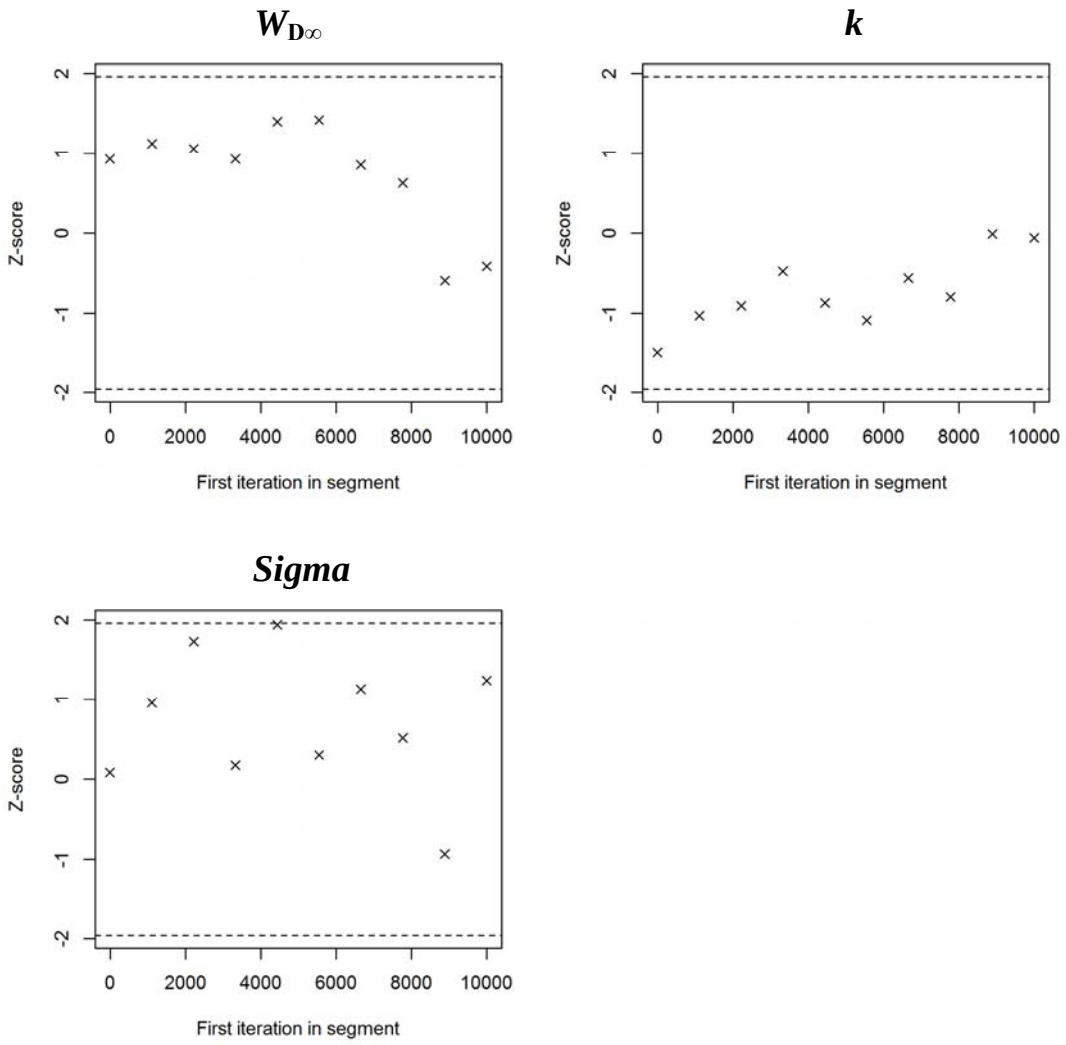
Species	W_{Dmax} (cm)	$W_{D\infty}$ (mean)	k (yr $^{-1}$) (mean)	Reference
Neotrygon annotata	24	24.8	0.255	Jacobsen & Bennett (2010)
Neotrygon picta	28	31.8	0.1	Jacobsen & Bennett (2010)
Dasyatis pastinaca	57	121.5	0.089	Ismen (2003)
Dasyatis chrysonota	75	72.3	0.1225	Cowley (1997)
Himantura astra	80	77.3	0.0885	Jacobsen & Bennett (2011)
Dasyatis lata	100	130.4	0.13	Dale & Holland (2012)
Dasyatis dipterura	122	77.3	0.075	Smith *et al.* (2007)



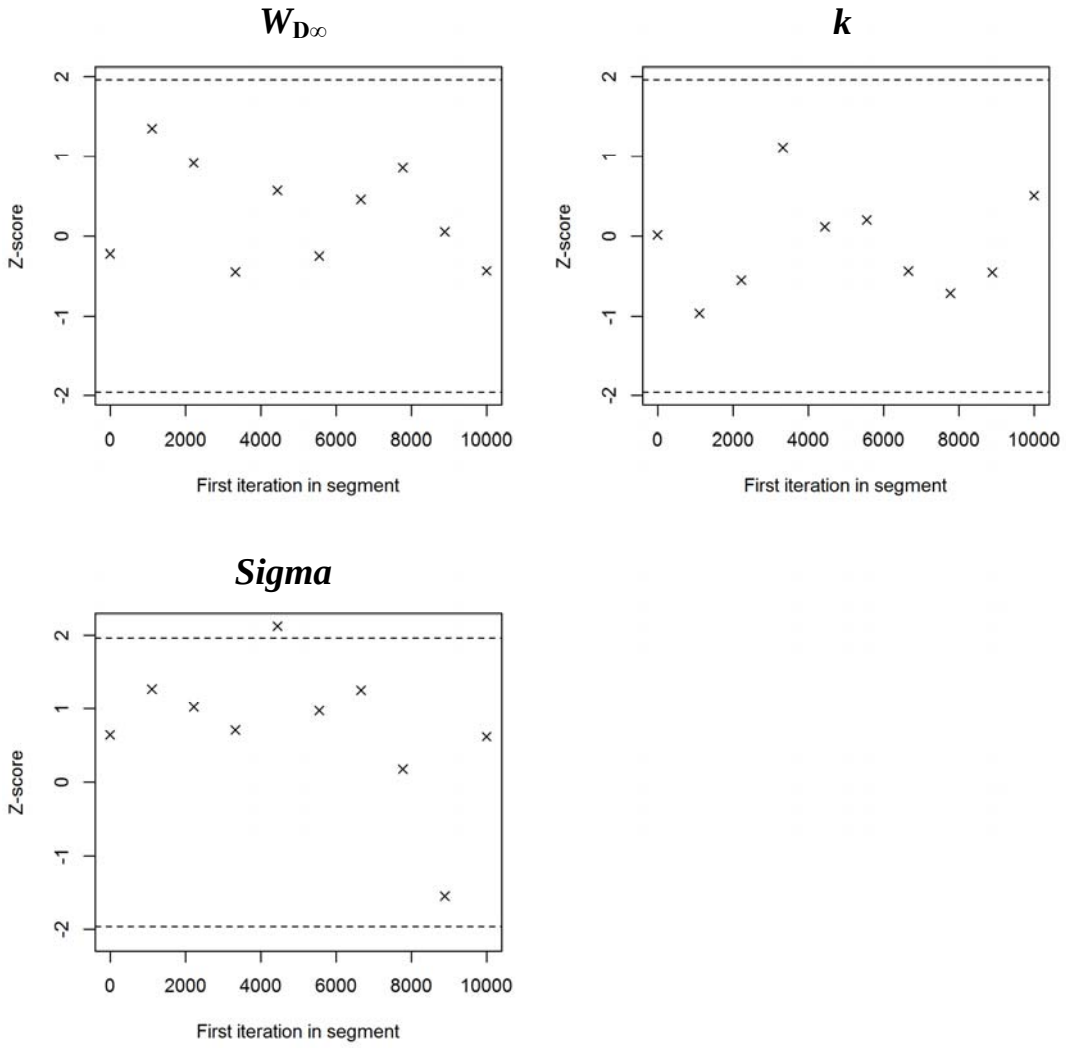
Supplementary Figure 3.1: Prior probability density distributions for growth parameters $W_{D\infty,}$ k and variation *sigma*



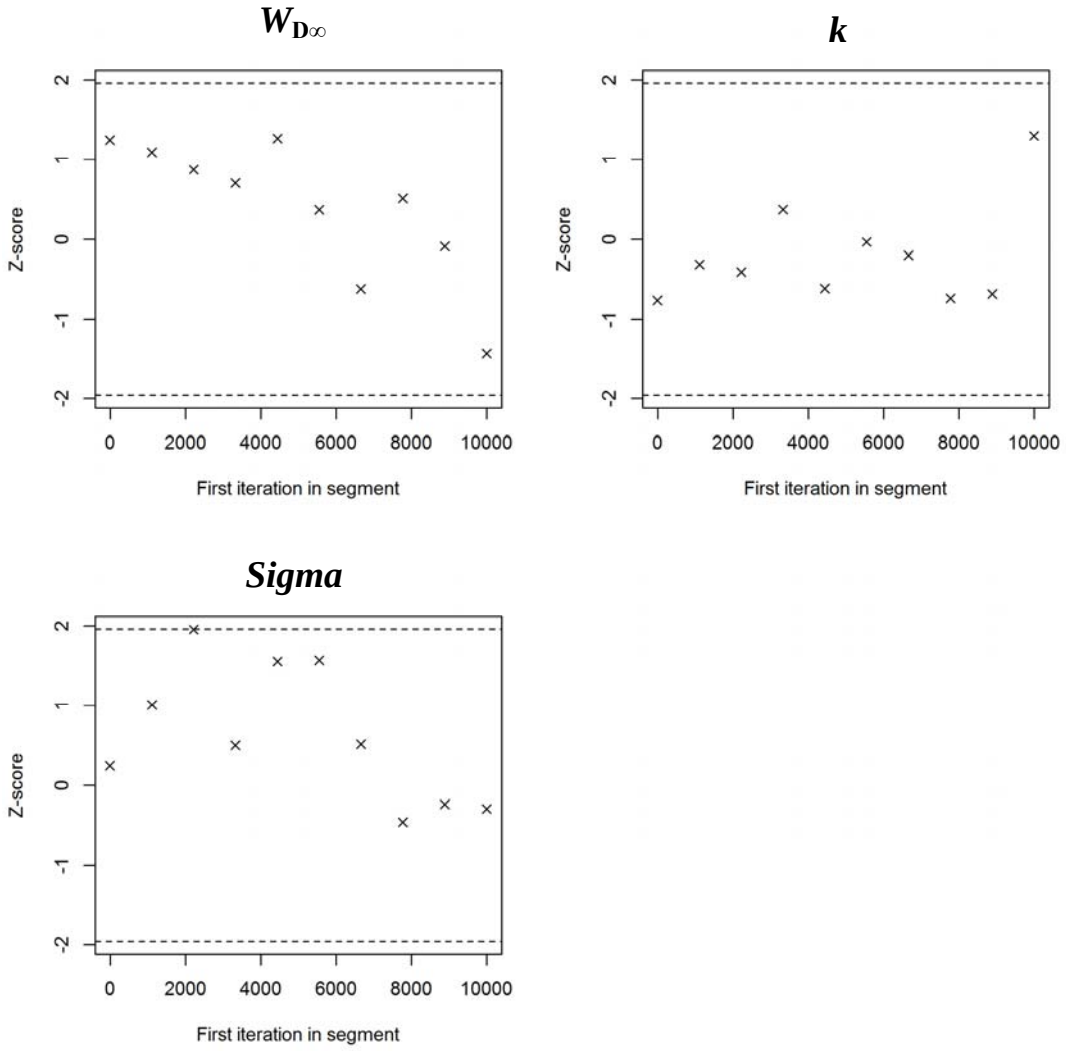
Supplementary Figure 3.2: Geweke's diagnostic test (Geweke 1991) for *Himantura uarnak*. Comparing means from the first 10% and the second 50% of the Markov Chain tests the convergence of mean estimates



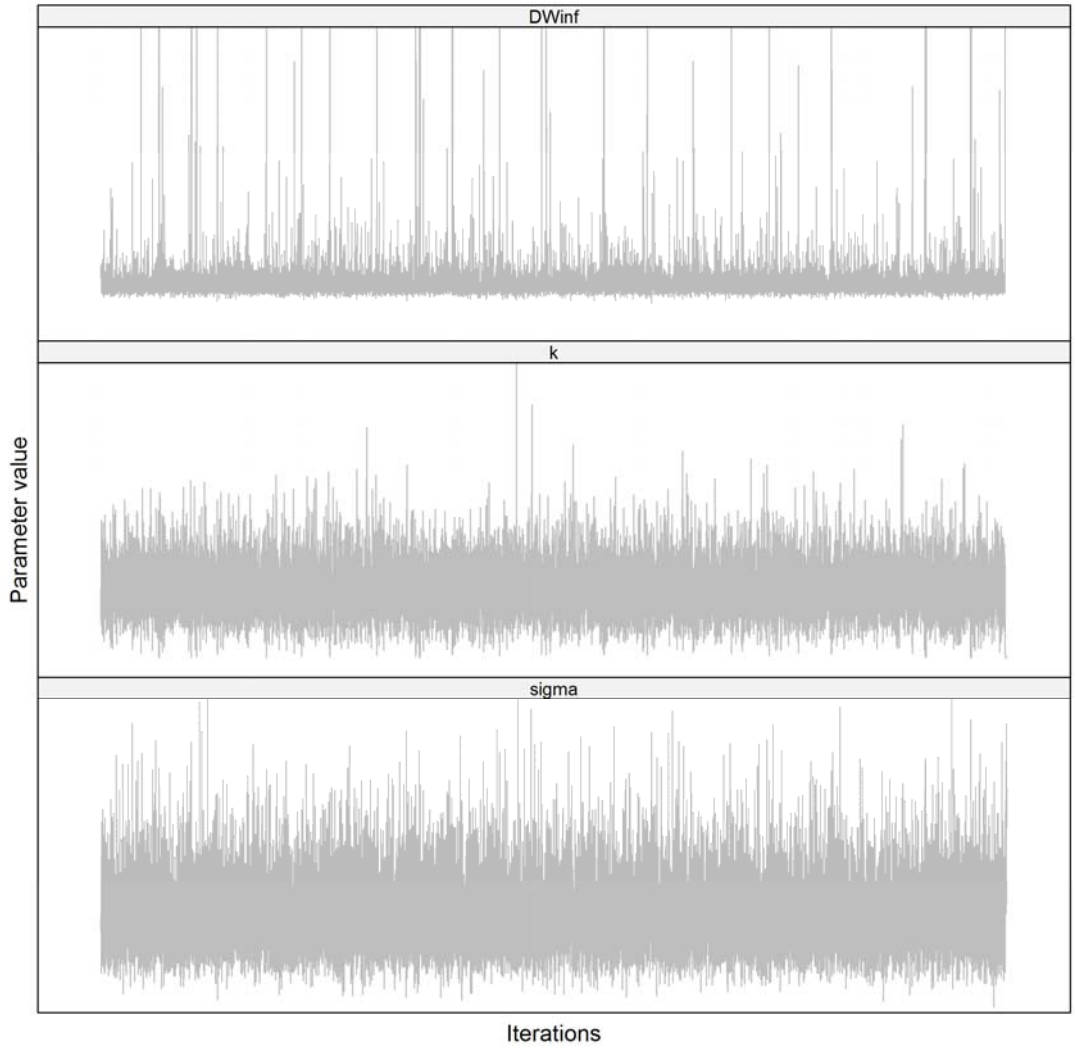
Supplementary Figure 3.3: Geweke's diagnostic test (Geweke 1991) for *Neotrygon kuhlii*. Comparing means from the first 10% and the second 50% of the Markov Chain tests the convergence of mean estimates



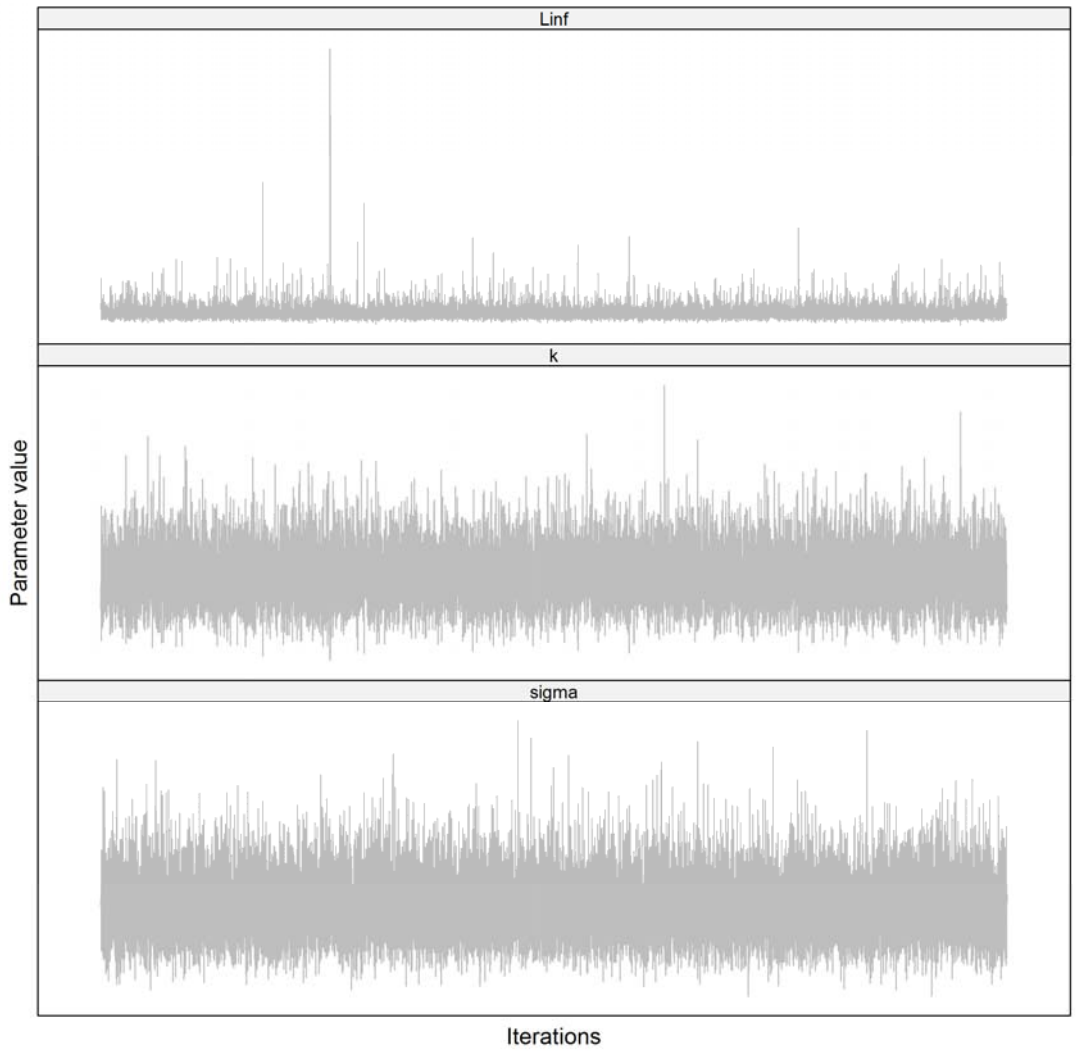
Supplementary Figure 3.4: Geweke's diagnostic test (Geweke 1991) for *Pastinachus atrus*. Comparing means from the first 10% and the second 50% of the Markov Chain tests the convergence of mean estimates



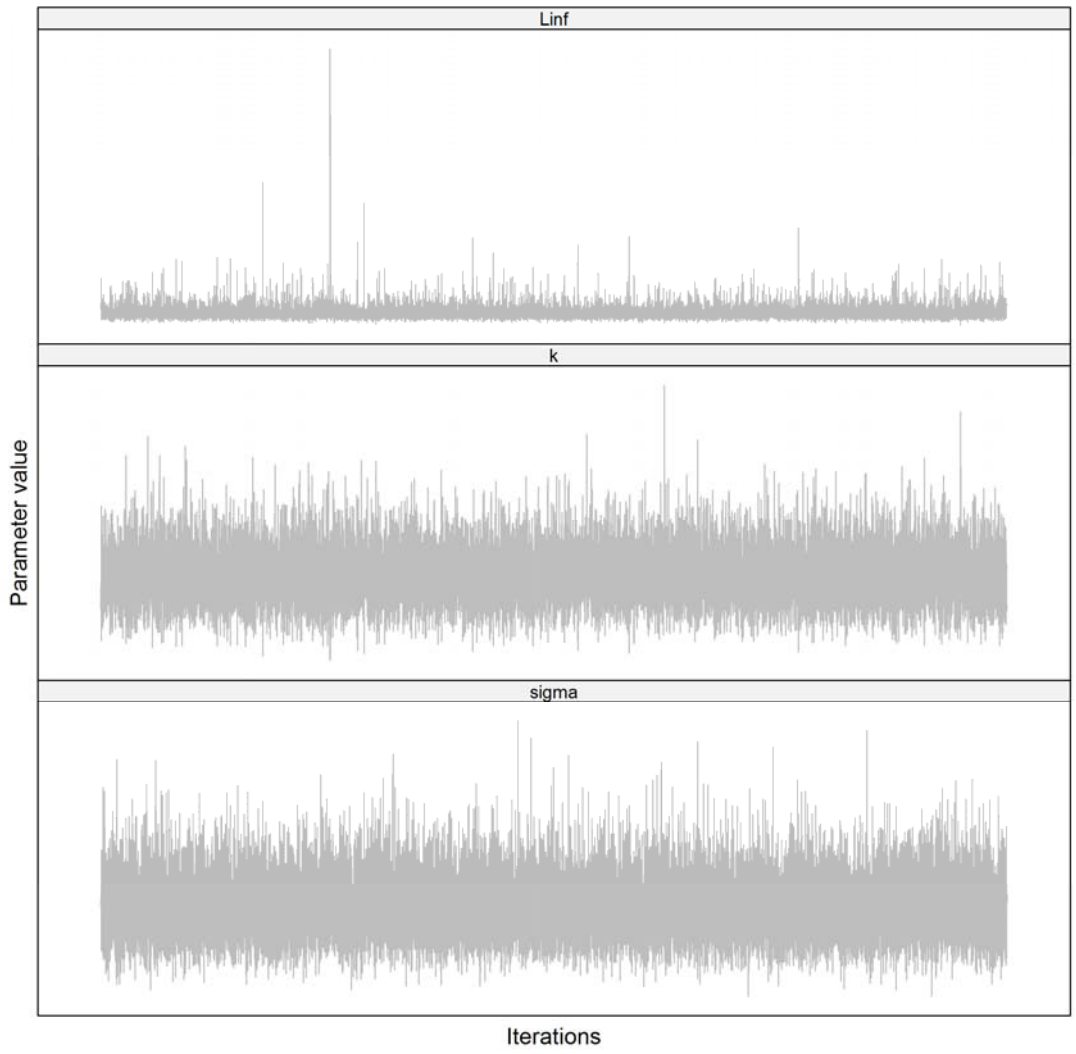
Supplementary Figure 3.5: Geweke's diagnostic test (Geweke 1991) for *Taeniura lymma*. Comparing means from the first 10% and the second 50% of the Markov Chain tests the convergence of mean estimates



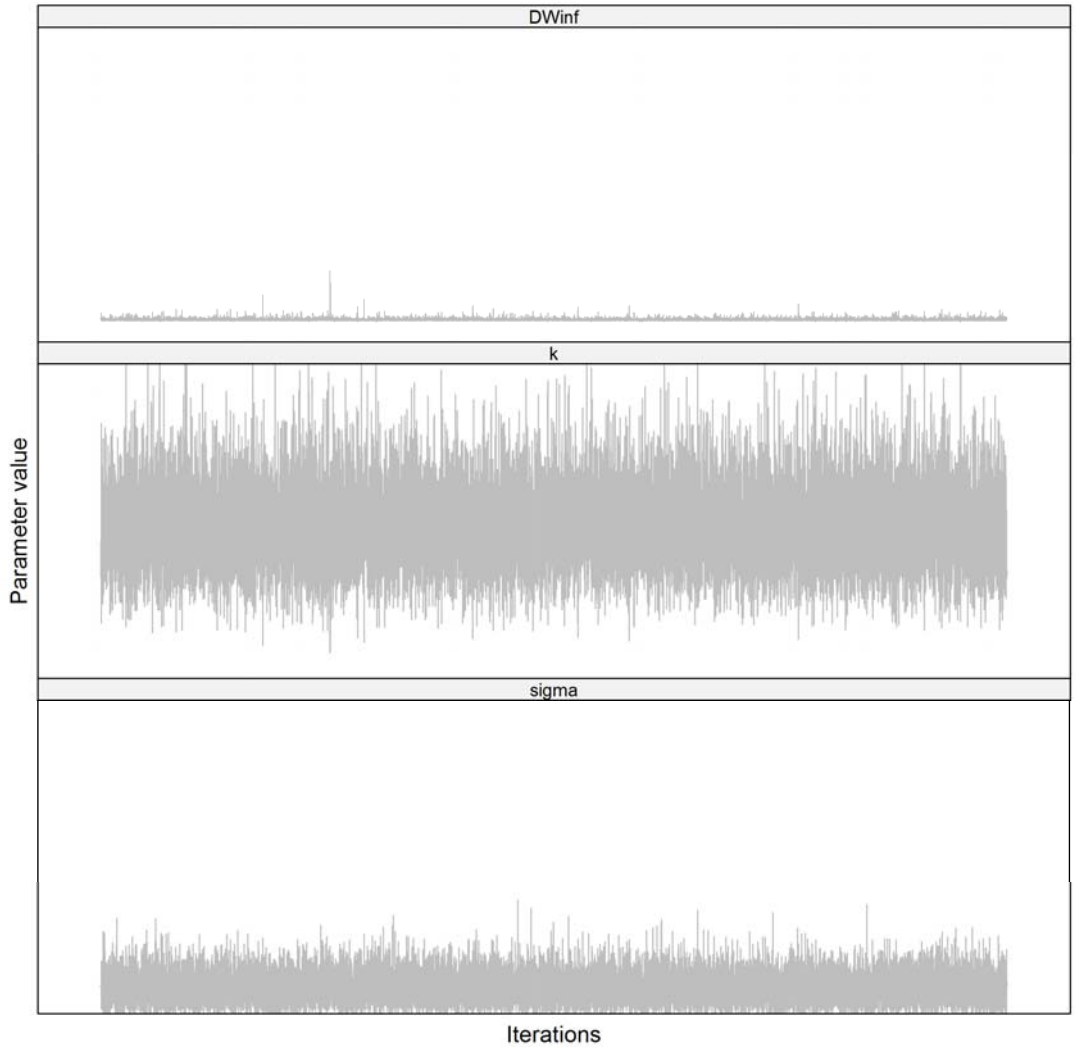
Supplementary Figure 3.6: Trace plots for for growth parameters $W_{D\infty,}$ k and variation *sigma* for *Himantura uarnak*



Supplementary Figure 3.7: Trace plots for for growth parameters $W_{D\infty}$, k and variation *sigma* for *Neotrygon kuhlii*



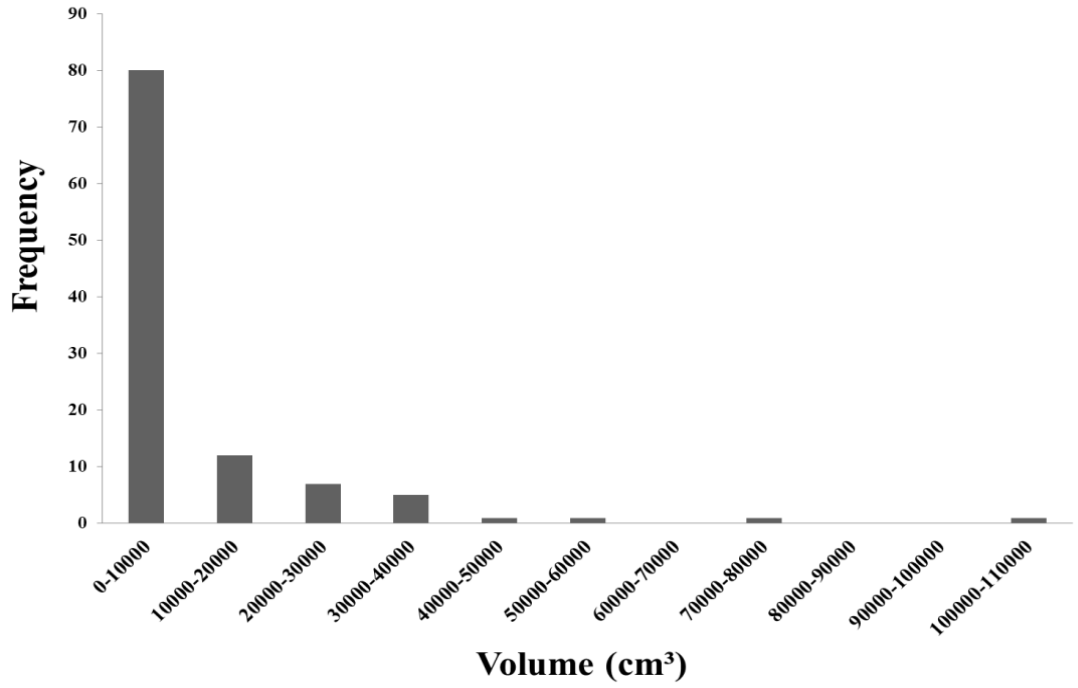
Supplementary Figure 3.8: Trace plots for for growth parameters $W_{D\infty}$, k and variation *sigma* for *Pastinachus atrus*



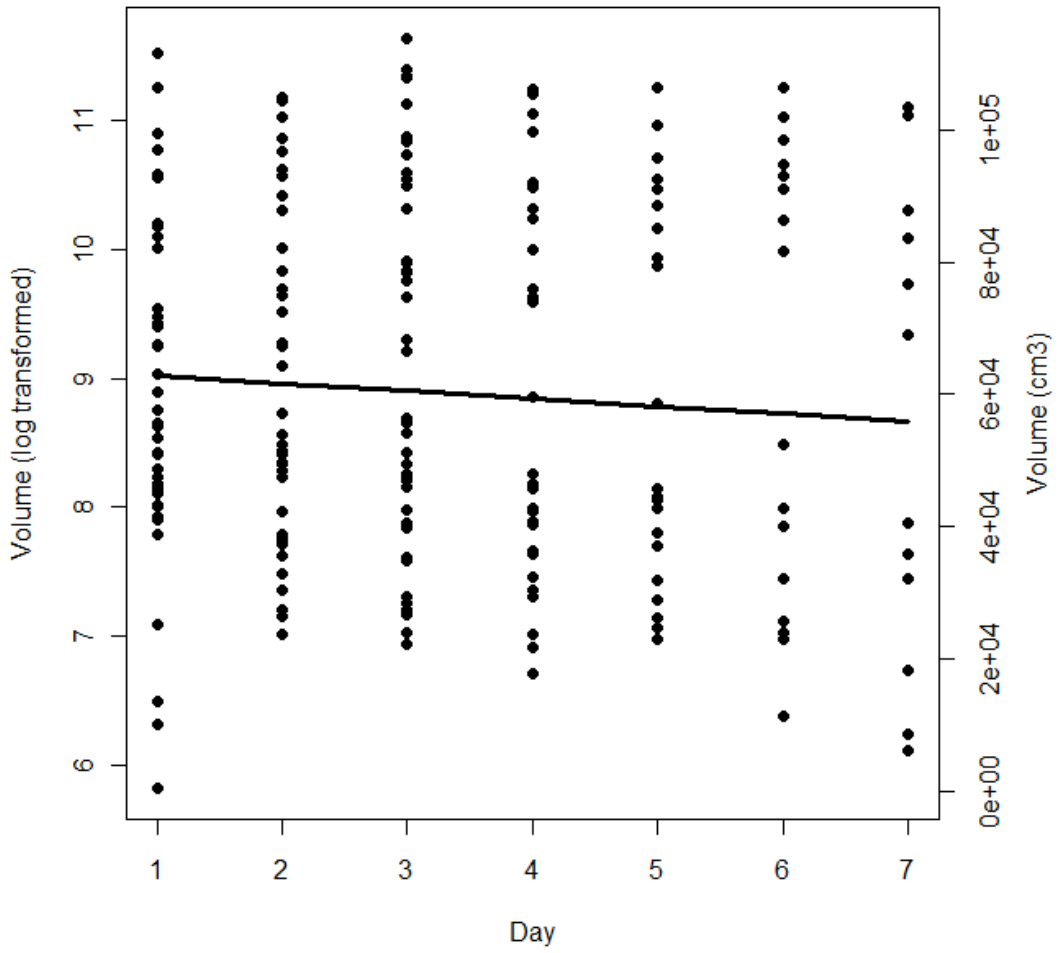
Supplementary Figure 3.9: Trace plots for for growth parameters $W_{D\infty,}$ k and variation *sigma* for *Taeniura lymma*

Appendix 2:

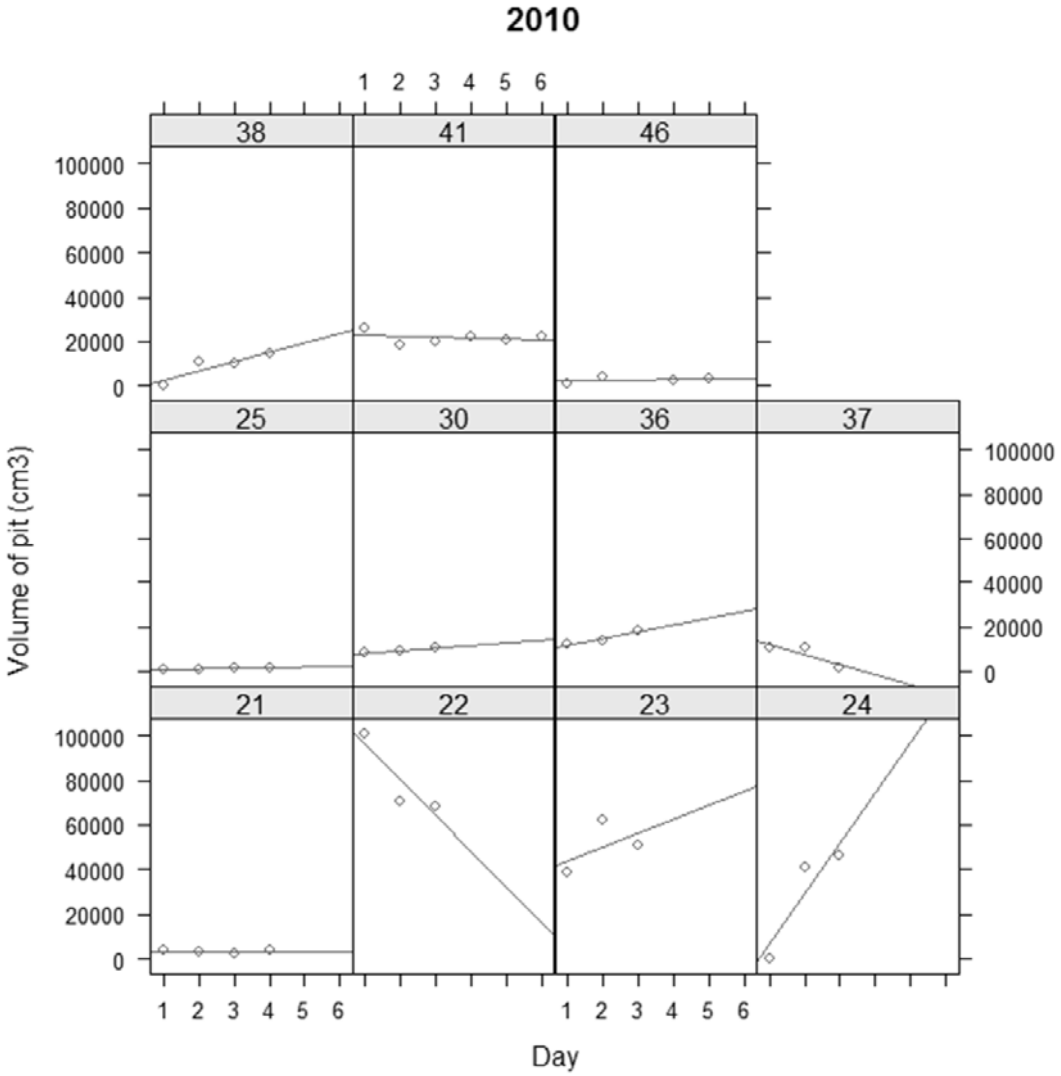
Supplementary Figures to Chapter 5



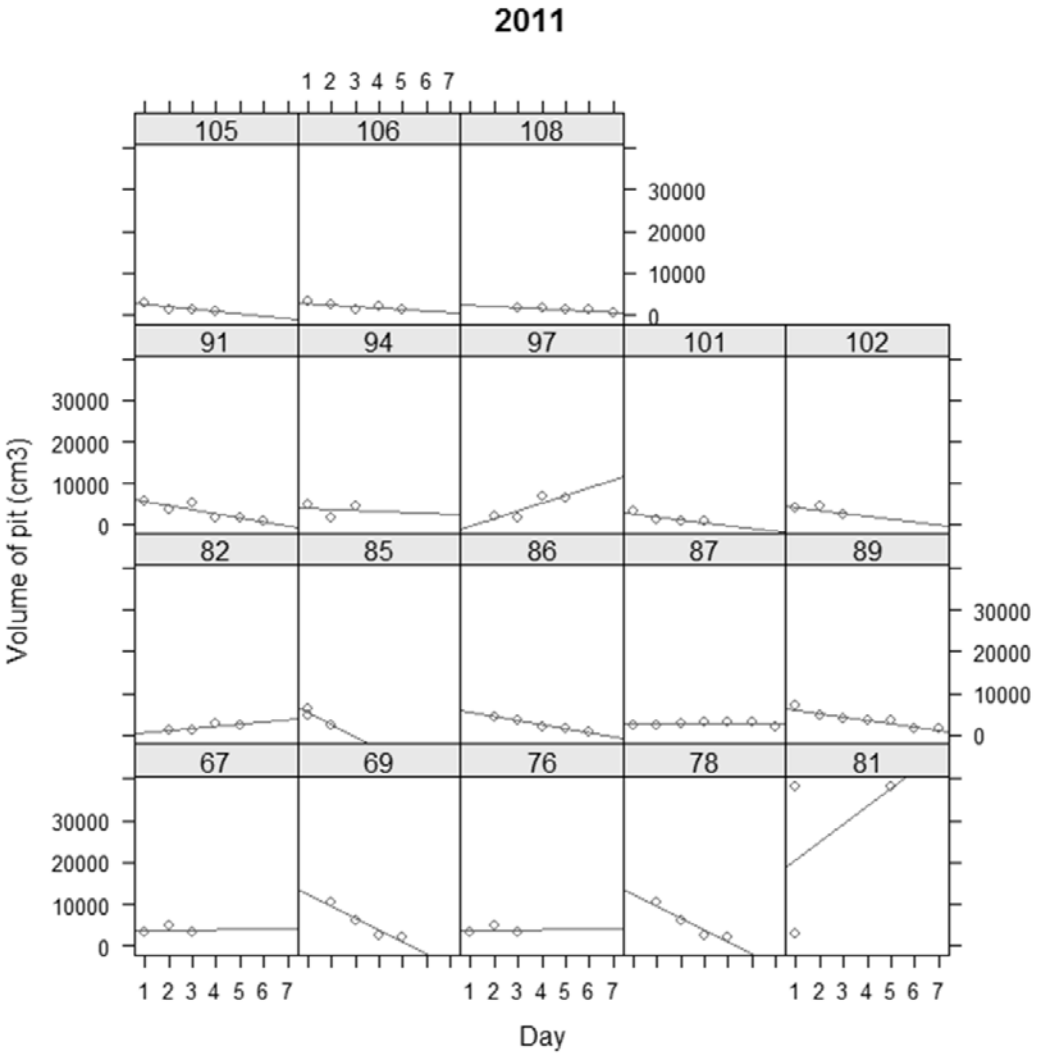
Supplementary Figure 5.1: Volume frequency of all pits sampled at Mangrove Bay



Supplementary Figure 5.2 The log-transformed volume of all pits sampled from 2009, 2010 and 2011 that were present for three days or more is plotted on the y-axis, against day of sampling on the x-axis. The un-transformed values are plotted on the opposite y-axis and the fitted line for the population of pits obtained by the linear mixed effects model is shown



Supplementary Figure 5.3: Linear relationships between day of sampling and volume of each of the pits sampled from September 2010 that were present for three days or more



Supplementary Figure 5.4: Linear relationships between day of sampling and volume of each of the pits sampled from February 2011 that were present for three days or more

Appendix 3:

Supplementary Tables to Chapter 6

Supplementary Table 6.1: Prey taxa identified to the highest possible taxonomic resolution from the stomachs of each ray species, quantified by dietary measures: Percentage frequency of occurrence (%FO) and percentage of numerical importance (%n). Prey category refers to the categories used to pool data for analyses

Appendix 3

Prey Group	Total n	Prey Taxa	n	%n	%FO	n	%n	%FO	n	%n	%FO	n	%n	%FO	n	%n	%FO
Annelids	**1,623**	**Archiannelida**	0	0	0	0	0	0	0	0	0	1	0.13	2.94	0	0	0
		Clitellata	0	0	0	0	0	0	0	0	0	0	0	0	3	0.53	9.09
		Oligochaeta	25	3.28	13.64	9	4.31	14.29	23	3.07	8.33	119	15.91	29.41	58	10.3	63.64
		Phyllodocida	20	2.62	13.64	10	4.78	10.71	0	0	0	89	11.9	23.53	14	2.49	27.27
		Polychaeta	15	1.97	22.73	24	11.48	14.29	58	7.75	12.5	13	1.74	5.88	1	0.18	9.09
		Sabellidae	0	0	0	0	0	0	5	0.67	4.17	7	0.94	5.88	2	0.36	18.18
		Spionidae	2	0.26	4.55	0	0	0	0	0	0	3	0.4	2.94	0	0	0
		Terebellida	0	0	0	0	0	0	0	0	0	1	0.13	2.94	0	0	0
		Unidentified Annelida	109	14.3	27.27	128	61.24	64.29	435	58.16	54.17	130	17.38	35.29	319	56.66	45.45
Bivalves	**139**	**Bivalvia**	49	6.43	31.82	5	2.39	7.14	38	5.08	12.5	37	4.95	23.53	10	1.78	18.18

Appendix 3

Brachyurans	**226**	**Brachyura**	123	16.14	63.64	9	4.31	10.71	79	10.56	29.17	3	0.4	8.82	12	2.13	36.36
Cephalopods	23	**Octopoda**	4	0.52	9.09	0	0	0	0	0	0	0	0	0	0	0	0
		Sepiida	10	1.31	18.18	0	0	0	0	0	0	0	0	0	0	0	0
		Teuthida	5	0.66	9.09	0	0	0	1	0.13	4.17	1	0.13	2.94	2	0.36	9.09
Copepods	6	**Copepoda**	0	0	0	0	0	0	0	0	0	6	0.8	5.88	0	0	0
Fishes	14	**Heteroconger sp.**	9	1.18	13.64	0	0	0	0	0	0	1	0.13	2.94	0	0	0
		Teleostei	3	0.39	9.09	1	0.48	3.57	0	0	0	1	0.13	2.94	0	0	0
Gastropods	12	**Gastropoda**	4	0.52	4.55	0	0	0	4	0.53	8.33	4	0.53	5.88	0	0	0
Other prawns	229	**Eucarida**	0	0	0	0	0	0	0	0	0	23	3.07	14.71	0	0	0
		Euphausiidae	25	3.28	13.64	1	0.48	3.57	0	0	0	8	1.07	5.88	0	0	0
		Amphipoda	1	0.13	4.55	3	1.44	3.57	0	0	0	9	1.2	14.71	1	0.18	9.09
		Stomatopoda	46	6.04	59.09	16	7.66	32.14	40	5.35	29.17	24	3.21	20.59	32	5.68	36.36

Penaeid prawns	483	**Penaeidae**	309	40.55	63.64	3	1.44	7.14	57	7.62	29.17	20	2.67	20.59	94	16.7	45.45
Priapulids	49	**Priapulidae**	3	0.39	4.55	0	0	0	8	1.07	4.17	23	3.07	11.76	15	2.66	36.36

Supplementary Table 6.2: Summary of infauna identified from sediment cores.

Prey Group	Class	Order	Family	Taxa	n
Annelids	Polychaeta			Unidentified	434
			Teribellidae		36
			Glyceridae	*Glycera* spp.	347
			Sabellidae		945
Chaetognaths					7
Telesots				*Thalassoma* Spp.	3
Cnidaria	Anthazoa	Alcyonacea			10
Crustaceans				Unidentified	1
	Malocostraca	Decapoda	Penaeidae		30
	Malocostraca	Decapoda		(infraorder) Brachyura	3
	Ostracoda				5
Echinodermata	Asteroidea				2
	Echinoidea				3
	Ophiuroidea				11
	Clypeastroidea				111
Mollusca	Gastropoda			Unidentified	945
			Cypraeoidae		10
			Cerithiidae		14
			Patellidae		13
			Nassariidae		14
			Trochidae		9
	Bivalvia			Unidentified	192
				Siliqua lucida	7
			Solenidae		12
			Tellinidae		41
Platyhelminthes				Unidentified	7
	Cestoda	Cestodaria			3

Appendix 4:

Front cover of *Marine and Freshwater Reseach* Vol:63, Issue 3, 2012, highighting the research paper derived from chapter 5

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